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THE EXPERIMENTAL DEMONSTRATION THAT PERIARTERITIS NODOSA IS A MANIFESTATION OF HYPERSENSITIVITY

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The etiology of periarteritis nodosa has long been obscure, and has provoked a great deal of speculation. Widely differing views have been expressed, through the years, concerning the cause of this destructive vascular lesion. It has been attributed to syphilis (1); to a filterable virus (2); to a variety of different infections (3); to a single, but unidentified infectious agent (4); to "toxic injuries" (5); to disease of the central nervous system (6). Seventeen years ago Gruber (7) made the suggestion that it might be due to hypersensitivity. In line with that possibility, several writers have remarked on the suggestively frequent coincidence of asthma and periarteritis nodosa (8) (9) (10).

One of us (10, 13) has recently reported a series of cases in which typical, fresh lesions of periarteritis nodosa were found in patients who came to autopsy in this department shortly after having had serum sickness, or hypersensitive reactions to sulfonamides, and evidence was presented which strongly indicated that the vascular lesions were caused by the hypersensitive reactions. In a search of the scant literature on the pathology of serum sickness it was found that Clark and Kaplan (14) had noted the presence of similar lesions in the heart and testis of two patients who had had serum sickness shortly before death. These authors commented upon the resemblance of the vascular lesions to those of periarteritis nodosa, but felt that "inferences concerning the pathogenesis of the latter disease, which are based only on the morphological resemblance between the two, are of dubious value and are not included within the purpose of this report."

After the publication of our first paper on this subject (10), Dr. Gurth Carpenter called our attention to a paper that he published with Eason in 1937 on the treatment of rheumatic fever with antiscarlatinal

serum (22). One of the treated patients developed serum sickness and died after 19 days. At autopsy periarteritis nodosa was found, which was regarded as the probable cause of death. The authors discussed the question whether the periarteritis nodosa might have been related to the serum sickness, but they favored the view that the vascular lesions were more probably related to the rheumatic infection, and were only coincidental in relation to the serum sickness. They wrote: "We suggest there is no evidence that serum was in any way responsible for the death." At that time there was, indeed, little reason to regard the serum sickness as other than a coincidence unrelated to the periarteritis nodosa.

Ten years ago, Vaubel (11), in a study of the relation of hypersensitivity to the lesions of rheumatism, noted vascular abnormalities in the hearts of rabbits that had received repeated subcutaneous and intravenous injections of horse serum. He described the lesions as consisting of "hyperplasia of the intima, a fibrinoid swelling of the intima and media with splitting of the elastic fibers, and a proliferation of adventitial cells, histiocytes and connective tissue cells, mostly in nodular formations" which resembled the Aschoff bodies of rheumatism. These alterations were encountered in varying degree in 5 out of 46 serum treated rabbits. The affected vessels were limited to the heart. Vaubel stated that the vascular alterations bore a resemblance to periarteritis nodosa, to thrombangeitis obliterans, and "above all to rheumatic arteritis." Masugi and Sato (12), in an experimental study of the relation of hypersensitivity to nephritis, mentioned vascular alterations in the heart and liver in rabbits that had received repeated intravenous injections of horse serum. These alterations were reported in detail in separate papers. In the heart, they were described as consisting of a sub-intimal deposit of a fibrinoid substance, and fibrinoid swelling of the media with infiltration of basophilic, mononuclear cells, often with giant nuclei. The authors concluded, in agreement with Vaubel, that the vascular alterations resembled those of "rheumatic arteritis" (20). In the other viscera the arterial lesions were described as "very slight", consisting only of "swelling" of the vessel wall and intimal hyperplasia (25). Miura (28) described identical lesions, limited to the heart, in rabbits that were given injections of foreign serum. Metz (21) reported that he had produced lesions

similar to those of periarteritis nodosa by injections of horse serum into rats, but the numerous illustrations that he published as typical of his results provide no substantiation of his conclusions. The well-known high resistance of the rat to anaphylactic sensitization would render this animal a particularly unsuitable subject for the experiment. Most investigators have been unable to produce either anaphylactic shock or local anaphylactic tissue damage (Arthus phenomenon) in the rat. Rintelen (24) reported that in sensitized animals *local* vascular damage and inflammation could be produced by injecting foreign serum into the lumen of a segment of artery that was isolated from the circulation by a ligature at each end of the segment. This drastic procedure reproduces, at the most, the conditions of a local Arthus reaction, and throws no light on the possibility of vascular damage under the conditions of serum sickness. The same may be said of the experiments of Masugi and Sato (12), in which necrosis of renal arterioles was produced by the injection of foreign protein directly into the renal arteries of previously sensitized rabbits. It has long been known (15, 16) that vascular necrosis occurs in the Arthus phenomenon (*i.e.*, the local haemorrhage and necrosis occurring at the site of injection of foreign protein into the tissues of a highly sensitized animal). In the experiments of Masugi and Sato, 2–5 cc. of egg albumen, undiluted or mixed with gelatin, were injected directly into the renal artery of the previously sensitized rabbit. In one third of the animals the injection was repeated three times into the same artery. Examination of the kidneys of those of the animals that died shortly after the injection showed the injected material plugging the arteries and capillaries. Widespread renal infarction occurred in the animals that survived, and the arterioles associated with the infarcts were necrotic. The authors regarded the vascular damage produced by this procedure as similar to that in periarteritis nodosa, but they noted that the arteriolar necrosis was preceded by a massive infiltration of red blood cells into the media, an occurrence that does not precede the necrosis of the media in periarteritis nodosa. As stated above, this experiment, by bringing the concentrated antigen directly into contact with the renal arterioles by local injection into the renal artery of a previously sensitized animal, reproduces essentially the conditions of the Arthus phenomenon.

While the exceptional circumstances surrounding the last human case that we reported (13) provided excellent evidence that the development of periarteritis nodosa followed the hypersensitivity reaction, and while the conditions associated with the seven cases included in our first report (10) were such as to render it highly improbable that the presence of periarteritis nodosa was merely an accidental coincidence, still there could be no absolute exclusion of coincidence. Furthermore, since treatment with serum and sulfonamides is carried out in order to combat infection, our human cases were subject to the possibility (though the circumstances rendered it a highly improbable possibility) that the periarteritis nodosa might have resulted from the infection rather than from the serum or drug hypersensitivity reaction. The present study was undertaken, therefore, in order to determine whether periarteritis nodosa could be produced experimentally as a result of hypersensitivity, under conditions that would exclude the complicating factors of coincidence and infection. For this purpose we elected to attempt to reproduce the conditions obtaining in serum sickness. The above-mentioned vascular alterations described by Vaubel (11) and Masugi (20) in the rabbits that had received intravenous injections of horse serum were suggestive in this regard, but the lesions were confined to the heart, as was stated above, and the investigators regarded them as resembling rheumatic lesions more than periarteritis nodosa.

EXPERIMENTS

It was pointed out in our first paper on this subject (10) that the patients who developed periarteritis nodosa following serum sickness had received large amounts of serum, or injections of serum repeated at intervals throughout their illness; and that the two patients of Clark and Kaplan had likewise received large amounts of serum. Fleisher and Jones (17, 19) had described a febrile reaction and a cutaneous flush, particularly in the ears, in serum treated rabbits, which they regarded as manifestations comparable to serum sickness in man. The flush appeared in most of their rabbits on the fifth or sixth day after a single intravenous injection of a relatively large amount of horse serum. For these reasons, we used as the initial intravenous

dosage 10 cc. of sterile horse serum, without preservative,[1] per kilogram of rabbit.

Fourteen male albino rabbits, averaging 2200 grams in weight, were divided into two groups. Group A (9 animals) received horse serum only. Group B (5 animals) received the same dosage of horse serum, but, in addition, they were given sulfadiazine in order to reproduce the conditions obtaining in those of our reported human cases of periarteritis nodosa that had received both serum and sulfadiazine (10). Finestone and his co-workers (18) have reported that a daily dosage of 0.5 gm. of sulfadiazine per kilogram, administered for three weeks, produced no toxic symptoms in rabbits, and no histological alterations were found in the kidneys, which were the only organs examined. We used that dosage in the present experiments. The days on which the drug was given are shown in Table 1.

Beginning on the fifth day after the initial injection of serum, the rabbits were observed carefully for the detection of any cutaneous flushing, such as that described by Fleisher and Jones (17). The ears were examined with especial care. A definite flush was observed in the ears of rabbits 13, 14, 15, 16 and 21 of Group A, and in rabbits 7, 9, 10 and 11 of Group B. The flush was first observed, in the different rabbits, between the fifth and eighth day after the injection of the serum. It gradually faded, disappearing after two to three days.

By the fifth to the sixth day following the injection of the serum, the rectal temperature had risen sharply in all of the rabbits to a level of 104°F. to 105.8°F. The fever lasted for several days, gradually returning to the level normal for the rabbit (102°F.). It may be noted that there was no correlation between the rise in temperature and the occurrence or degree of flushing of the ears. Flushing occurred in rabbit 21, for example, the maximum temperature of which was 104°F., whereas no flush was observed in rabbit 17, the temperature of which reached 105.8°F. The maximum temperature of rabbits 12 and 16 was 104.6°F., but rabbit 16 developed an aural flush while rabbit 12 did not.

Twelve days after the first injection of serum, a skin test was per-

[1] The serum was kindly supplied by the Medical Research Division of Sharp and Dohme, Inc.

TABLE 1

*Arterial lesions in animals rendered hypersensitive**

RABBIT	9/5	9/16	9/17	9/22	9/24	10/1	10/2	10/8	10/9	ARTERIAL LESIONS†
				Group A: Serum only						
12	10 cc./kg. horse serum intraven.	Skin test 0.1 cc. serum intracut.	50 x 30 mm. oedema erythema	1 cc. serum intraven.	10 cc./kg. serum intraven; died from shock	—	—	—	—	Heart: nec; pv. inf; oed. Lung: nec; inf; pv. inf; oed. Testis: oed.
13	" "	" "	45 x 45 mm. oedema erythema haemorrhage necrosis	" "	10 cc./kg. serum intraven.	Skin test 0.2 cc. serum intracut.	50 x 35 mm. oedema erythema haemorrhage necrosis	2 cc. serum intraven.	Killed	Heart: nec; oed. Lung: nec; inf; pv. inf; oed. Liver: oed. Thymus: oed.
14	" "	" "	30 x 30 mm. oedema erythema	" "	" "	Killed	—	—	—	Heart: nec; inf; pv. inf. Liver: nec; inf; pv. inf; oed. Kidney: nec; pv. inf. Pancreas: pv. inf; oed. Adrenal: pv. inf; oed. Testis: pv. inf; oed.
15	" "	" "	30 x 30 mm. oedema erythema	" "	" "	Killed	—	—	—	Heart: oed. Liver: inf; oed.
16	" "	" "	45 x 45 mm. oedema erythema	" "	" "	Killed	—	—	—	Heart: nec; pv. inf; oed. Thymus: nec; pv. inf; oed. Testis: inf. pv. inf; oed. Liver: oed.
17	" "	" "	45 x 25 mm. oedema erythema haemorrhage	1 cc. serum intraven.; died from shock	—	—	—	—	—	Heart: nec; pv. inf; oed. Lung: nec; oed. Pancrease: nec; inf; pv. inf; oed. Testis; nec; pv. inf; oed. Liver: inf; pv. inf; oed.

											pv. inf; oed. Liver: inf; pv. inf; oed.
18	"	"	"	"	50 x 45 mm. oedema erythema	1 cc. serum intraven.	Killed	—	—	—	—	Heart: pv. inf; oed.
19	"	"	"	"	55 x 50 mm. oedema erythema haemorrhage necrosis	1 cc. serum intraven.; died from shock	—	—	—	—	—	Heart: nec; pv. inf. Lung: nec; inf; pv. inf; oed. Liver: nec; inf; pv. inf; oed. Pancreas: nec; inf; pv. inf: oed. Kidney: nec; inf; pf. inf; oed. Testis: nec; inf; pv. inf; oed.
21	"	"	"	"	45 x 30 mm. oedema erythema haemorrhage	1 cc. serum intraven.	10 cc./kg. serum intraven.	Skin test 0.2 cc. serum intracut.	40 x 25 mm. oedema erythema haem-morrhage necrosis	2 cc. serum intraven.	Killed	No arterial lesions found.
Group B: Serum and sulfadiazine. 0.5 gm./kg. of sulfadiazine by mouth per day 9/5 to 9/15 and 9/22 to 10/1												
7	"	"	"	"	45 x 30 mm. oedema erythema	"	"	Killed	—	—	—	—	Spleen: nec; pv. inf; oed. Kidney: pv. inf; oed. Testis: pv. inf; oed.; Heart: oed.
8	"	"	"	"	45 x 25 mm. oedema erythema	"	"	10 cc./kg. serum intraven.	Killed	—	—	—	Heart: nec; pv. inf; oed. Liver: nec; inf; pv. inf; oed. Kidney: nec; inf; pv. inf. Pancreas: nec; inf; pv. inf. Spleen: nec; inf; pv. inf. Testis: nec; inf; pv. inf.
9	"	"	"	"	75 x 40 mm. oedema erythema haemorrhage necrosis	"	"	1 gm. Na. sulfadiazine intraven.	Killed	—	—	—	No arterial lesions found.

TABLE 1—(*Concluded*)

RABBIT	9/5	9/16	9/17	9/22	9/24	10/1	10/2	10/8	10/9	ARTERIAL LESIONS
10	10 cc./kg. horse serum intraven.	Skin test 0.1 cc. serum intracut.	35 x 25 mm. oedema erythema	1 cc. serum intraven.	10 cc./kg. serum intraven.	Killed	—	—	—	Heart: nec. pv. inf. Liver: nec; inf; pv. inf; oed. Testis: nec; inf; pv. inf. Adrenal: nec; pv. inf. Kidney: pv. inf.
11	" "	" "	75 x 35 mm. oedema erythema	" "	1 gm. Na sulfadiazine intraven.	Killed	—	—	—	Lung: nec; inf; pv. inf; oed.

* See text for occurrence of erythema and fever following the serum injections.

† Nec. = necrosis of media. Inf. = infiltration of media and/or intima with inflammatory cells. Pv. inf. = adventitial and perivascular infiltration with inflammatory cells. Oed. = oedema of media.

formed on all of the rabbits. All were found to have become hypersensitive at this time. The skin reactions were of the prompt, anaphylactic type. Oedema was present within half an hour. This hypersensitivity that resulted from the single, large, sensitizing injection of serum was of a high degree. As can be seen in table 1, the skin reactions, though developing promptly, enlarged progressively, appearing on the following day as large, elevated, erythematous lesions with, in some cases, a central area of haemorrhage and necrosis. These were typical Arthus reactions.

On the seventeenth day following the first injection, all of the rabbits were given an intravenous injection of 1 cc. of horse serum. The serum was injected very slowly and, in spite of the high degree of hypersensitivity as shown by the skin tests, this procedure was survived by most of the animals with no apparent symptoms. Only 2 of the rabbits (nos. 17 and 19) died from anaphylactic shock. One other (no. 12) became ill and prostrated immediately after the injection, but soon recovered.

Two days later one rabbit from each group (nos. 7 and 18) was killed. All of the remaining rabbits of Group A, and rabbits 8 and 10 of Group B were given intravenously an amount of serum equal to that of the initial dose, *i.e.*, 10 cc. per kilogram. The serum was injected very slowly, and only one rabbit (no. 12) died from shock. The others bore the injection with no apparent symptoms. Rabbits 9 and 11 of Group B were given no horse serum at this time but, as may be seen in Table 1, the daily dosage of sulfadiazine, which had been discontinued for a week, was started again.

Within 1 to 6 days following the last injection of serum the ears of all of the remaining animals of Group A and of the two animals of Group B that had received the serum (nos. 8 and 10) became flushed. All of the animals of Group A developed fever (103.4°F. to 107°F.) following this injection. The temperature of both of the rabbits 8 and 10 of Group B rose to a maximum of 104.8°F. The daily temperature reading of the two rabbits of this group that were given sulfadiazine but no serum at this time (nos. 9 and 11) showed no elevation above normal during the following week.

Twenty-six days after the first serum injection, and seven days after the last injection, all of the remaining animals were killed, except nos.

13 and 21 of Group A. On that day these two rabbits were given a skin test with 0.2 cc. of horse serum. Both developed large, typical Arthus reactions, with haemorrhage, necrosis and sloughing. One week later they were given a third intravenous injection of serum, slowly administered, this time 2.0 cc. There was no reaction, and they were killed on the following day.

When the various animals died or were killed during the experiment, autopsies were performed immediately. The tissues were fixed in Zenker-formol and in formalin for histological study.

Examination of the histological sections showed beautiful arterial lesions, typical of periarteritis nodosa. Every stage of the process encountered in human periarteritis nodosa could be found in the various rabbits, and in the majority of the animals all stages were present in different arteries in the same rabbit. The simplest lesion consisted in oedema of the media, which spread the muscle fibres widely apart. More advanced lesions consisted in necrosis, fibrinoid alteration and hyalinization of the media, with infiltration of inflammatory cells which were predominantly mononuclear but included polymorphonuclears. Many of these polymorphonuclears had prominent, markedly eosinophilic granules. Unfortunately, the confusing appearance of the rabbit's pseudo-eosinophils in histological sections rendered it hardly possible for us to evaluate properly the degree of infiltration by true eosinophils. Marked adventitial and perivascular infiltration with inflammatory cells, predominantly mononuclear, was present, and in some instances there was, in addition, necrosis and fibrinoid alteration of the adventitia. The intima of many vessels was infiltrated with mononuclear cells, proliferation of the intimal endothelium was present in many of the vessels, and some of them were occluded. Elastic tissue stains showed disappearance of the elastic fibrils in the necrotic portions of the media. In lesions of longer duration the necrotic vascular wall was replaced by connective tissue. The lesions were widespread throughout the body. The sites in which affected vessels were found, and the character of the lesions in each affected organ of the different rabbits are shown in Table 1. Figures 1 to 15 illustrate representative lesions, which will be recognized as being altogether like the lesions of periarteritis nodosa in man. As is the case in human periarteritis nodosa, some of the affected vessels show

necrosis of their entire circumference, while in others only a segment of the circumference is necrotic.

DISCUSSION

In the present study, periarteritis nodosa has been reproduced in all of its essential details in the experimental animal by creating conditions similar to those obtaining in serum sickness in the human being. It is reasonable to believe that the aural flush and the rise in body temperature that occur in rabbits at the end of the first week following the injection of foreign serum are analogous to serum sickness in man, as was pointed out some years ago by Fleisher and Jones and their coworkers (17, 19). The latter investigators made no mention of having examined the tissues of their animals. It seems altogether probable that the vascular alterations seen by Vaubel (11) and by Masugi and his coworkers (20) in the hearts of rabbits that had received repeated intravenous injections of foreign serum were of the same nature as those in our rabbits, though these investigators regarded them as resembling "rheumatic arteritis" more than periarteritis nodosa. The widespread, markedly destructive vascular lesions produced in the present experiments exhibit all of the characteristics of periarteritis nodosa. We have not encountered actual aneurysm formation, but that is lacking in many cases of human periarteritis nodosa. However, in many vessels the condition necessary for aneurysm formation (*i.e.*, necrosis of a segment of the artery, with complete destruction of the muscle and elastic tissue) is beautifully shown. It is, indeed, rather remarkable that aneurysm formation or rupture has not occurred in vessels such as those illustrated in Figs. 4 and 7. Had hypertension been present there is little doubt that the totally necrotic segment of the wall would have given way. The other local complication of periarteritis nodosa which occurs in some cases of the disease in man, *i.e.*, infarction from vascular occlusion, has been encountered only once in our animals. In rabbit 10 there was an infarct in the liver involving a number of lobules with the associated bile ducts in the periportal spaces. Affected branches of the hepatic artery contained organizing thrombi.

The demonstration that periarteritis nodosa is a manifestation of anaphylactic hypersensitivity is in line with the long recognized fact

that the blood vessels are susceptible to anaphylactic injury. The anaphylactic wheal is, itself, a result of capillary damage producing increased permeability. The haemorrhage at the site of injection of a foreign protein to which the body has been highly sensitized (Arthus phenomenon) is clear evidence of a more marked vascular injury. Gerlach (23), long ago, described necrosis of arterioles at the site of the Arthus phenomenon, and the observation has been repeatedly confirmed. However, most of the haemorrhage in the Arthus phenomenon comes from damaged capillaries and minute terminal arterioles. According to our experience, a primary necrosis of the larger arteries of the size of those that are prominently affected in periarteritis nodosa rarely, if ever, occurs in the Arthus reaction. This may be due to the fact that when the foreign protein is injected into the tissues it cannot diffuse readily into the muscular wall of the vessel, and this particularly when the tissue fluids contain a high titre of precipitin which will combine with the antigen, as is the case in the Arthus sensitized body. It is not improbable that the damage to the media of the arteries in periarteritis nodosa may be a consequence of damage to the endothelium of the intima, analogous to the familiar capillary endothelial damage in local anaphylactic reactions. There is ample evidence of endothelial damage both in human periarteritis nodosa and in our experimental animals. Accumulation of fluid and inflammatory cells beneath the endothelium of affected arteries, and actual necrosis of the endothelium, is present. Indeed, the earliest lesion in the media (*i.e.*, localized oedema, widely separating the muscle fibers) is analogous to the cutaneous anaphylactic wheal (Fig. 10), and it may be that the subsequent death of the muscle fibres is due to interference with their supply of nutritive materials and oxygen, which is normally derived from the blood circulating through the lumen, and which can be markedly reduced when the fibres are widely separated by an accumulation of oedema fluid. The arterial lesions, both in their focal distribution and in their origin as foci of localized oedema, strongly suggest that periarteritis nodosa represents, in effect, "hives" of the blood vessels, analogous to the cutaneous hives familiar in serum sickness and in generalized anaphylactic reactions produced by other foreign antigens.

It is of particular interest that the present experiments show that marked lesions of periarteritis nodosa can result from *a single large*

injection of foreign serum in a previously non-hypersensitive body, and that repeated injections after hypersensitivity has developed are not necessary for the production of the vascular necrosis. Vaubel (11) stated that vascular lesions could not be produced in rabbits by a single large injection (30 cc.) of foreign serum. He used only 2 animals, however, and does not state how long he waited after the injection before killing them. It is ordinarily stated in treatises on hypersensitivity that repeated injections are necessary in order to establish a degree of sensitivity that will permit an animal to exhibit an Arthus reaction. As was stated in the body of this paper, following a single large injection of serum our animals developed the capacity to react to the small intracutaneous test dose of 0.1 cc. of serum with large, persisting lesions which, in five of fourteen animals, were haemorrhagic, and in four necrotic, *i.e.*, Arthus type reactions (see Table 1). The early experiment of Longcope (27), in which rabbits that had been given injections of horse serum on three successive days developed the capacity to exhibit Arthus type reactions, is comparable immunologically to the situation of a single large dose, as practised in our experiment. Rabbits 17 and 19 of Group A demonstrate that a single large injection of foreign serum can be sufficient to produce periarteritis nodosa. Each of these rabbits received approximately 20 cc. of horse serum. Twelve days later the skin tests showed that they had developed a very high degree of hypersensitivity, both exhibiting Arthus type reactions. One week later they were given 1.0 cc. of serum intravenously, and both died promptly from anaphylactic shock. Histological study of their tissues showed widespread periarteritis nodosa. The vascular necrosis and the adventitial, medial and intimal infiltration with inflammatory cells (Figs. 4, 5, 6, 7, 8, 9) make it clear that the damage had been done before the injection which caused the animals' sudden death from anaphylactic shock. When a single small amount of serum is administered, the degree of hypersensitivity that it brings into being is less than that caused by a large amount. Furthermore, in the case of the large initial dose, an abundance of antigen will be present in the body while the antibody titre rises, and the opportunity for the production of vascular lesions as a result of the interaction of antigen and antibody will be correspondingly prolonged.

This fact that a single, large dose of serum can cause periarteritis

nodosa, and that subsequent injections after hypersensitivity has become established are not necessary for the production of the vascular lesions, is of some importance in relation to the therapeutic administration of antisera. It may be noted that the patients in our previous report (10) who developed periarteritis nodosa in association with serum sickness had received quite large amounts of serum, and this was true of the cases of Clark and Kaplan (14).

Examination of table 1 makes it clear that there are individual differences in the degree to which different rabbits, treated in the same manner, develop the vascular lesions. The lesions in rabbit 14, for example, were much more severe and widespread than those of rabbit 15 which had received precisely the same treatment. Furthermore, the differences in individual susceptibility appear to outweigh the factor of dosage of serum (*cf.*, for example, rabbit 17 and rabbit 21 of Group A). We have not been able to correlate these individual differences satisfactorily with differences in the evidences of the degree of hypersensitivity (aural flush, fever, skin reactivity). The precipitin titre was not studied. Similar differences in the degree and extent of the vascular lesions are familiar in the case of periarteritis nodosa in the human being. In some cases only a single affected vessel in a single organ is encountered; in others, widespread lesions are present. Individual differences in the tendency to develop serum sickness are likewise familiar in the human being. Of a group of persons receiving the same amount of foreign serum, some will develop severe serum sickness, some mild reactions, and some no reaction at all.

The present experiments tell us nothing of the potentiality of sulfonamides to sensitize and produce periarteritis nodosa in the rabbit. Sulfadiazine was given to the animals in Group B because it was thought that the combination of horse serum and the drug might possibly be more effective in producing the vascular lesions than serum alone, since most of the patients in our preceding report (10) had received both serum and sulfonamide. Table 1 shows that this was not the case, and suggests that the vascular lesions observed in the animals of Group B were caused solely by the hypersensitivity to the serum, as the lesions in the animals of Group A undoubtedly were. It was pointed out earlier in this paper that when the administration of sulfadiazine was resumed in the case of rabbits 9 and 11 of Group B,

after having been discontinued for a week, no evidence of a hypersensitive reaction (no flush or fever) occurred, whereas the reinjection of serum into animals 8 and 10 of this group at the same period of time resulted in aural flushing and fever. Experiments are in progress in the attempt to produce in the rabbit periarteritis nodosa as a result of hypersensitivity to sulfonamides alone, comparable to the effect that we have described in man (13).

Is is of interest that *acute diffuse glomerulo-nephritis* occurred in 7 of the 9 rabbits that received horse serum only, and in 3 of the 5 animals that received both serum and sulfadiazine. The nephritis had the histological characteristics of acute glomerulo-nephritis in man. There was condensation of the glomerular tufts, with syncytial proliferation of the glomerular epithelium, haemorrhage in Bowman's capsule and in the tubules, and albumin and casts in the tubules (Fig. 16). In some cases there were small colloid droplets in the tubular epithelium. In Group A these various lesions were present in marked degree in rabbits 16 and 19; they were present to only a moderate degree in rabbits 13, 14, 17, 18 and 21; and they were absent in rabbits 12 and 15. It should be stressed that these glomerular lesions are entirely independent of the focal inflammatory lesions familiar in the kidneys of many rabbits. It can be seen from Table 1 that the occurrence of nephritis was not limited to animals that developed periarteritis nodosa of the renal arteries. The attack was upon the glomeruli independent of the arterioles. Longcope (26), thirty years ago, reported pioneering experiments in which glomerular lesions of the same general nature were produced by repeated injections of foreign protein into sensitized animals. Miura (28) described an increase in the nuclei of glomerular loops, which he designated as a "slight nephritis", in rabbits that had been given rather large doses of foreign serum intramuscularly. Masugi and Sato (12) produced glomerular lesions in rabbits by intensive treatment with repeated intravenous injections of horse serum. Most of their illustrations show large accumulations of a hyaline material in the glomerular loops, strongly suggesting amyloid. No amyloid stain was made. It is well known that amyloid can be produced in animals by repeated injections of foreign protein, though the rabbit produces amyloid less readily than the mouse. Nevertheless, there is one illustration in which

haemorrhage into a Bowman's capsule is shown, and erythrocytes and red cell casts were found in the urine. It seems altogether likely that the glomerular lesions in our rabbits are of the same nature as the fresh glomerular lesions described by Longcope and attributed by him to anaphylactic hypersensitivity. The "clinical" manifestations of this experimental nephritis are being studied.

CONCLUSIONS

1. Typical diffuse periarteritis nodosa has been produced experimentally by establishing in rabbits a condition analogous to serum sickness in man.

2. These experiments demonstrate that periarteritis nodosa is one manifestation of the anaphylactic type of hypersensitivity.

3. The clinical and pathological evidence, presented in our previous papers, that periarteritis nodosa has developed in patients as a result of hypersensitive reactions following foreign serum and sulfonamide therapy, supported by the present experiments, shows that widely different types of sensitizing antigens are capable of causing periarteritis nodosa in man, and suggest the advisability of attempting to discover and to eliminate the responsible antigen in each case diagnosed clinically.

4. The continued administration of foreign serum or sulfonamides after a hypersensitive reaction has occurred, or the injection of a single, large amount of foreign serum, increases the danger of producing the vascular damage by prolonging the contact of the sensitized body with the offending antigen.

5. Acute diffuse glomerulo-nephritis occurred in a number of the animals that developed a hypersensitive reaction to the foreign serum. This supports the view that some cases of glomerulo-nephritis in man may be due to hypersensitivity.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

FIG. 1. Hepatic artery of rabbit 14. Upper right segment of media destroyed; perivascular and medial infiltration with inflammatory cells.

FIG. 2. Low magnification of large hepatic artery of rabbit 8. Necrosis of media; infiltration of media and dense perivascular infiltration with inflammatory cells.

FIG. 3. Hepatic artery of rabbit 8. Destruction of media with fibrinoid and hyaline alteration; perivascular inflammatory infiltration.

FIG. 4. Renal artery of rabbit 19. The wall is totally necrotic except for the segment at the left. Marked medial and perivascular inflammatory infiltration; intimal infiltration and proliferation.

FIG. 5. Artery of renal pelvis of rabbit 19. Necrosis of half the circumference. Inflammatory infiltration of the necrotic segment of media and of the related adventitia; intimal proliferation.

FIG. 6. Hepatic artery of rabbit 19. Total destruction of media, which is replaced by hyaline-fibrinoid material. Inflammatory infiltration; proliferation of intima almost occluding lumen.

FIG. 7. Pancreatic artery of rabbit 19. Necrosis of all of media except segment at right. Inflammatory infiltration of necrotic portion of media, of adventitia and intima; intimal proliferation.

FIG. 8. Artery adjacent to testis of rabbit 17. The media is almost totally destroyed. Inflammatory infiltration of intima, media and adventitia.

FIG. 9. Pancreatic artery of rabbit 17. Widespread necrosis of media. Inflammatory infiltration of intima, media and adventitia.

FIG. 10. Artery of renal pelvis of rabbit 7. The left half of the illustrated segment of this artery is normal. The right half shows marked oedema of the wall tearing the muscle fibers apart. A large, sharply localized segment of the wall was distended with oedema in this manner, appearing as an urticarial wheal of the vessel wall. In early lesions of this type inflammatory infiltration has not yet appeared.

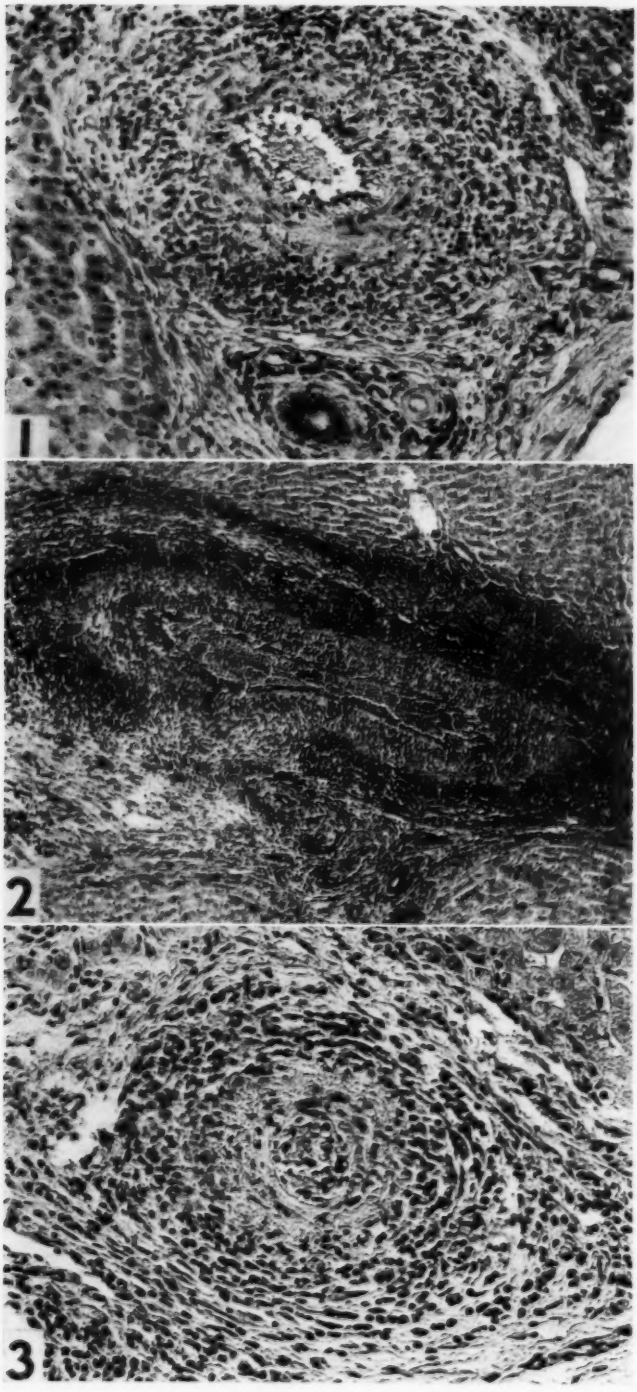
FIG. 11. Artery adjacent to adrenal of rabbit 10. Intimal proliferation; much of media destroyed and undergoing reparative fibrosis; adventitial and perivascular inflammatory infiltration.

FIGS. 12 AND 13. Hepatic arteries of rabbit 10. These are more advanced lesions, in which the completely (Fig. 12) or almost completely (Fig. 13) destroyed media has been replaced by connective tissue.

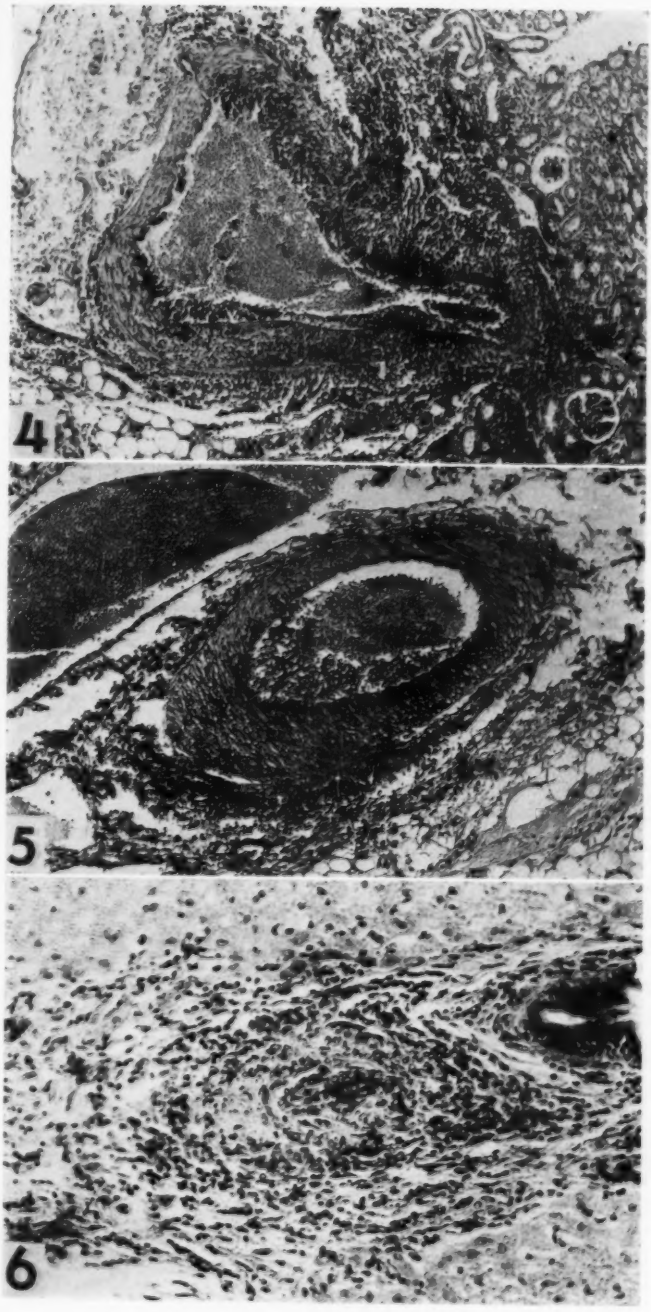
FIG. 14. Artery from thymus of rabbit 16. The media has been partly destroyed and replaced by connective tissue. The lumen is narrowed by intimal proliferation of connective tissue. Perivascular inflammatory infiltration.

FIG. 15. Pulmonary artery of rabbit 11. Half of the circumference destroyed; intimal proliferation; perivascular inflammatory infiltration.

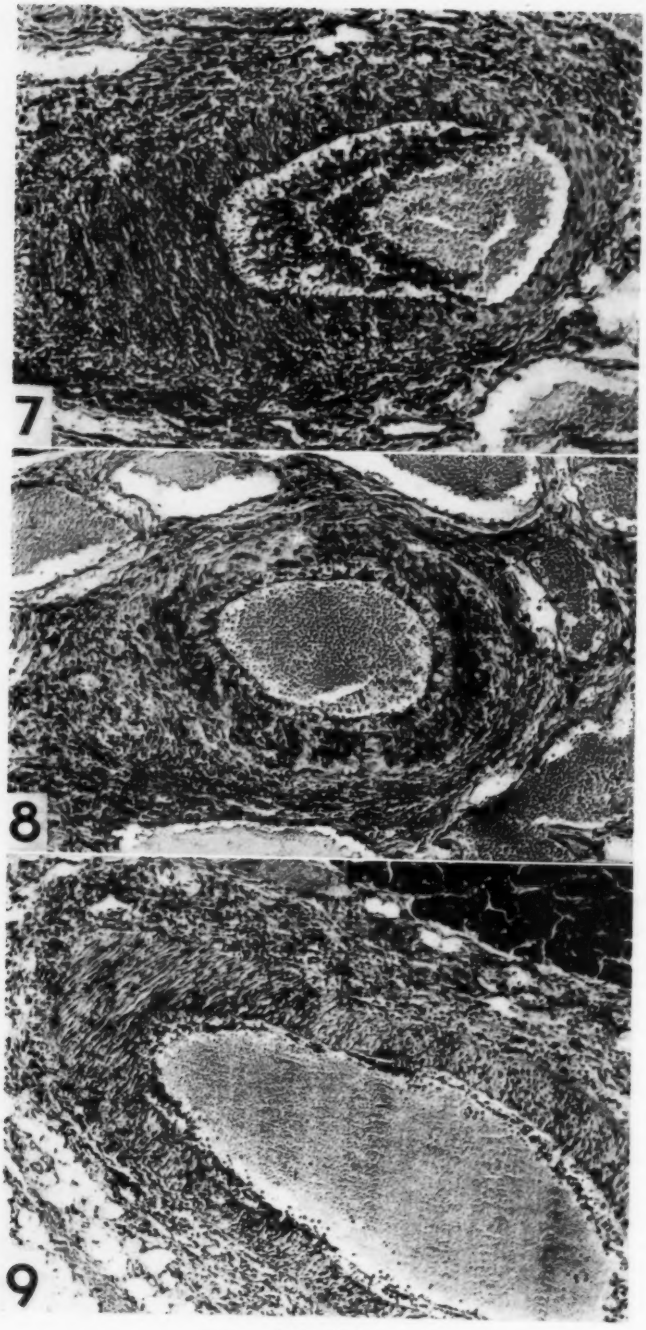
FIG. 16. Acute diffuse nephritis in rabbit 16. Syncytial proliferation of epithelium of glomerular tuft; coagulated protein in Bowman's capsule and tubules; the question-mark shaped tubule at the right is filled with red blood cells.



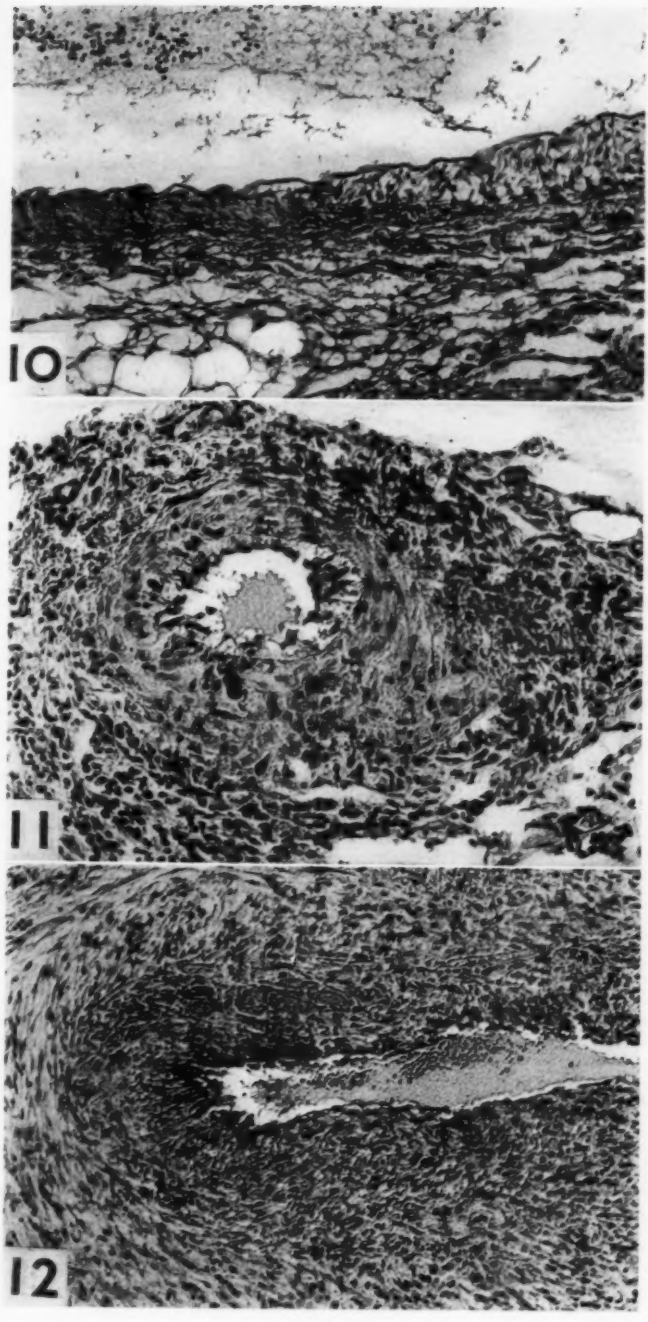
FIGS. 1–3



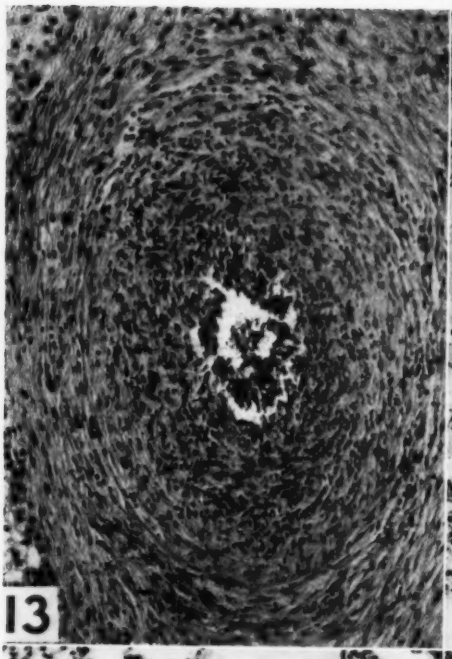
FIGS. 4–6

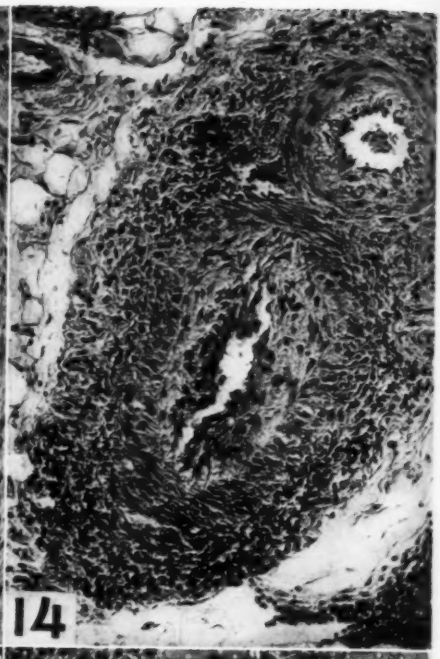


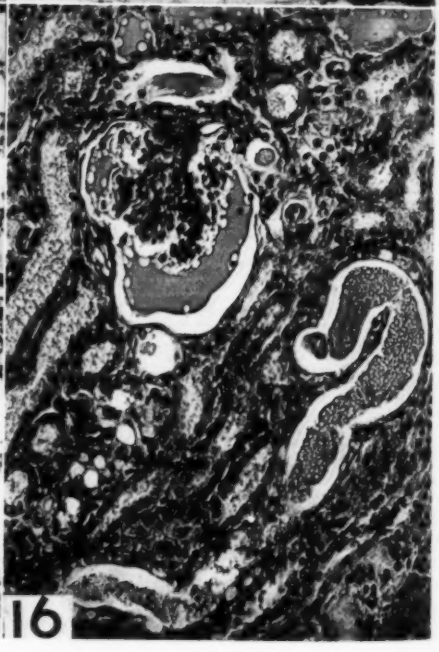
FIGS. 7–9



FIGS. 10–12







FIGS. 13–16

AN EPIDEMIC OF ACUTE RESPIRATORY DISEASE ASSOCIATED WITH ATYPICAL PNEUMONIA

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During the past several years ever increasing interest has been shown in a respiratory disease recently termed "primary atypical pneumonia, etiology unknown" (1). Several other names, including "virus pneumonia", have been used in describing this disease. In 1935 Bowen (2) described a disease which he called "acute influenza pneumonitis." Allen (3) recorded 68 cases of "acute pneumonitis" out of a series of 2,081 cases of respiratory disease in 1936. Clinical and pathological descriptions have been given by Kneeland and Smetana (4), Longcope (5–6), Dingle and Finland (7), and Gallagher (8). That the disease may vary in severity is well known. Bock (9), in going over 1,667 cases of acute respiratory infection treated at the Stillman Infirmary of The Harvard University during the period from September 1935 to March 1938, found 52 cases of pneumonia, the great majority of which were believed to represent a virus pneumonia. These cases on admission showed symptoms similar to the usual type of respiratory infection accompanied by fever. Roentgen-ray examination on the fourth or fifth day after admission to the Infirmary gave the first evidence of lung involvement. Reimann and Havens (10) made an association between mild upper respiratory infection and atypical pneumonia. This observation has not been generally supported in the literature. That this disease may be very contagious and reach epidemic proportions has been shown by Kornblum and Reimann (11), Daniels (12), and Adams et al (13). Some evidence that a filterable virus is the etiological agent of this disease has been brought out by Weir and Horsfall (14) and Stickney and Heilman (15).

An epidemic of primary atypical pneumonia, etiology unknown was observed in a boys' camp in Maine during the summer of 1942. At the same time there were numerous cases of respiratory disease varying from a mild common cold without fever to the severe disease picture of atypical pneumonia, except that no lung involvement was demon-

strated. It is believed that there was a common etiological agent present in most of the cases in which the respiratory disease was accompanied by fever and very likely, also, in many of the cases in which symptoms of common cold without fever existed.

DESCRIPTION OF THE CAMP

The camp, located by a lake in the State of Maine, included 100 individuals. 73 of these were boy campers, 24 were staff members, and 3 were wives of staff members. The personnel was stable with the exception of three boys who came into camp late in the season, one boy who left in the middle of the camp period because of homesickness, and the patients who required care outside of the camp. All of the others remained in camp during the eight week period from July 2nd to August 27th, 1942. During the summer occasional canoe trips were taken by groups from the camp but only infrequently did any boy visit inhabited parts of the surrounding country. For the most, the boys and the councillors had been associated with Eastern private schools, either as teachers or as students, during the preceding year. The boy campers varied in age from 7 through 14 years. The other group included individuals between the ages of 15 and 45 years. All of the boys and most of the councillors slept in two dormitories. Individual dormitory rooms, of which there were four, were occupied by boys of approximately the same age level; councillors were placed irregularly throughout these rooms. The boys and the staff had their meals together in a common dining hall. The camp infirmary was in a separate building from the rest of the camp. The boys and councillors shared modern toilet equipment and no interchange of towels was allowed.

CLINICAL MATERIAL

Councillors estimated that from 60 to 80 individuals out of the camp group of 100 suffered with cough during the eight week period. 35 patients with respiratory disease and fever were confined to bed. Of this group, 15 developed moist rales heard over the lung fields and of this latter group, 7 showed definite roentgenographic evidence of atypical pneumonia.

Cases of Pneumonia

There were 7 patients in this group. On the whole, the different patients showed about the same symptoms. A dry cough or a little coryza usually preceded the period of fever by a few days. Malaise, anorexia, weakness, nausea, and vomiting frequently accompanied the fever. The cough, unproductive at first, became more severe and in a few of the cases it was productive of a muco-purulent discharge after several days. One patient had a very severe headache along with the fever. Two individuals complained of pain in the ear and showed congestion of the tympanic membrane. There was very little complaint of sore throat and only slight injection of the pharynx was observed. Moist rales were heard over the lung fields, usually after several days. Sibilant rales were heard in two cases. Two others developed transient friction rubs. A little cyanosis was observed in only one case. Respiratory rate was not especially elevated but there was slight shallowness and hesitancy in breathing. The pulse infrequently exceeded normal by more than 20 to 35 beats to the minute. Except in one patient who developed a temperature of 105.4 degrees on the second day after admission, the rise in temperature was gradual over a period of two or three days to 102.6–104 degrees. Evening temperatures were usually about two degrees higher than morning temperatures. Roentgenograms were taken usually after several days and showed mottled areas and clouding in the lung fields. The white blood count was generally low or slightly elevated during the first few days. Late in the disease this usually showed a moderate rise. Three patients had short relapses lasting a few days. Sulfathiazole and sulfadiazine were used in these cases and except in one instance, these drugs appeared to have no effect on the outcome of the disease.

Case 1. A. F., a ten year old boy, was admitted to the camp infirmary on July 22, 1942, complaining of cough, coryza, and fever. He had lived in a country home during the month of June and he had no known contacts with individuals having cough or pneumonia just previous to his arrival in camp. During this time, however, he did play in a barn in which there were pigeons. He had had whooping cough at four years, measles at seven years, and chicken pox at eight years of age. He arrived at camp on July 2nd and on the following day a routine physical examination did not show any abnormality. From July 7th to July 10th he went on a camping trip with eleven others from camp. At least two individuals had cough

during this time. Of this group of 12 persons, six developed respiratory disease with fever between July 22nd and August 9th. On July 22nd this boy's temperature reached 100.2 degrees and he was put to bed. General physical examination was negative except for fever, moderate cough, slight coryza, and a slightly injected throat. On the following day he felt worse and complained of a little dyspnea. A transient sibilant rale was heard. On the third infirmary day no abnormal signs were made out over the lungs, although the temperature reached 102.4 degrees. The white blood count was 8,600. On July 25th the cough, which had been unproductive, now brought up colorless phlegm. The patient complained of some sore-

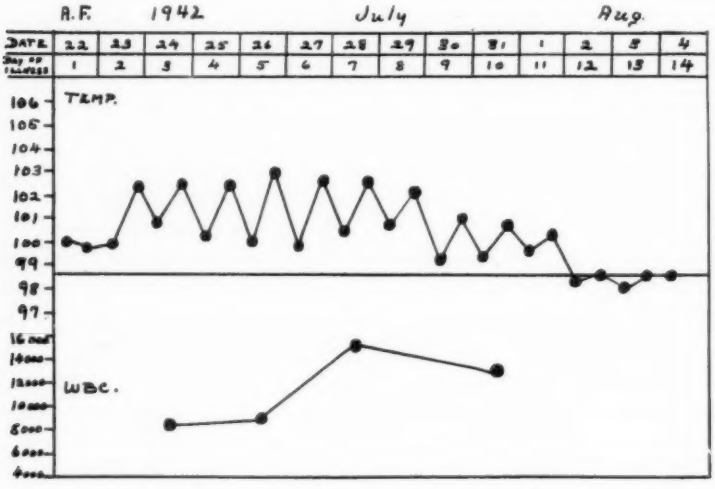

CHART 1. CASE 1. BOY AET. 10. TEMPERATURE CHART AND CURVE OF TOTAL LEUCOCYTES

Note moderate leucocytosis late in the illness

ness in the left side of the chest. The left ear pained him somewhat. Fine to coarse rales were heard over the right middle lung field posteriorly with some harshness of the breath sounds but no dullness. Roentgenograms showed a few scattered mottled areas in both lung fields with a clouded area about the right hilus radiating outward. The leucocytes were 8,900 of which polymorphonuclear neutrophils were 60 per cent, lymphocytes were 37 per cent, and juvenile neutrophils were 3 per cent. Sulfathiazole was used three days and sulfadiazine for three subsequent days but there was no dramatic improvement, although the temperature did gradually return to normal at the end of the six days. On the sixth day of fever there was noticeable increase in coughing and difficulty in breathing. Vomiting occurred

during this period. The white blood count increased to 15,300 on the seventh day and was 13,360 on the tenth day. The temperature returned to normal on the

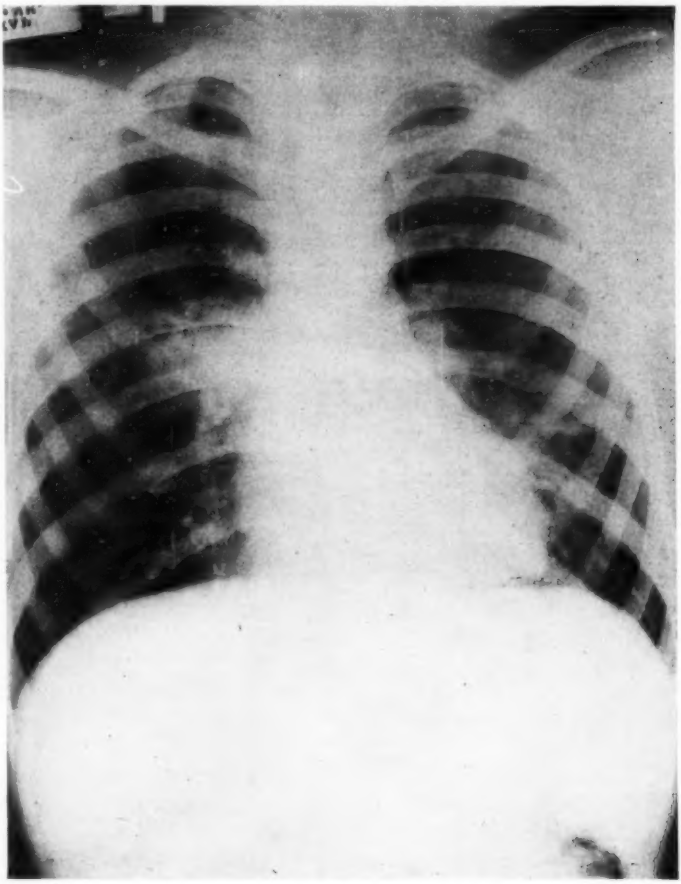

FIG. 1. CASE 1. ROENTGENOGRAM OF CHEST SHOWING AREAS OF INFILTRATION IN MID PORTION OF RIGHT LUNG AND LEFT LOWER LOBE ON JULY 25, 1942, THE FOURTH DAY OF ILLNESS

eleventh day at which time loud and sticky rales were heard over the lungs. The lungs were entirely clear by the fifteenth day.

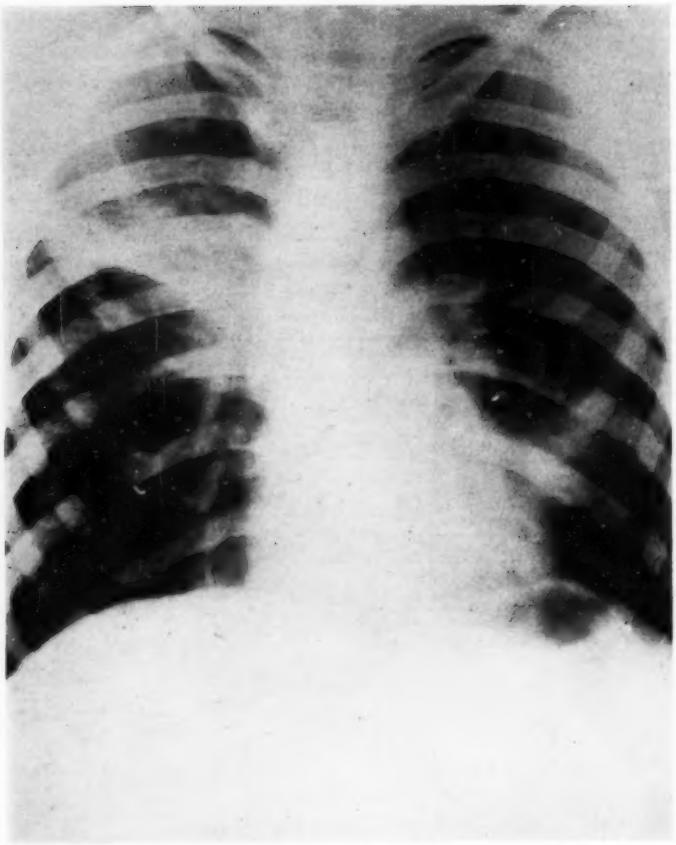

FIG. 2. CASE 1. ROENTGENOGRAM OF CHEST SHOWING MORE EXTENSIVE INFILTRATION, ESPECIALLY ALONG INTERLOBAR FISSURE TO THE RIGHT, ON JULY 28, 1942, THE SEVENTH DAY OF ILLNESS

Cases of Respiratory Disease with Fever and without Roentgenographic Evidence of Pneumonia

In this group are 28 cases. Individuals showed respiratory disease varying from cough with fever lasting two days to a severe illness without radiographic evidence of lung involvement with the fever lasting

as long as 17 days. One case showed symptoms fully as severe as some of those having atypical pneumonia, yet roentgenograms taken on the third and sixth days of illness showed no definite pulmonary shadows.

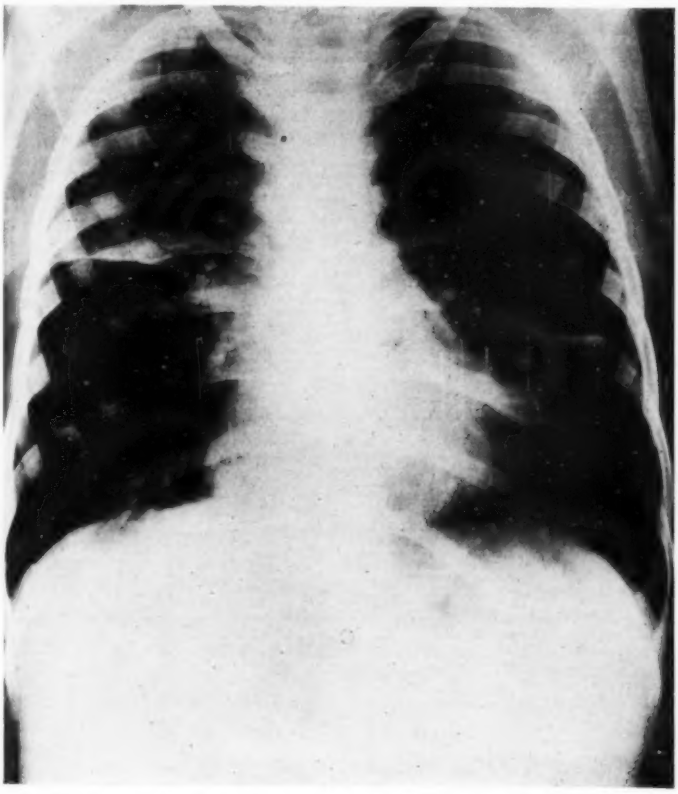

FIG. 3. CASE 1. ROENTGENOGRAM OF CHEST SHOWING PARTIAL CLEARING OF INFILTRATION ON AUGUST 3, 1942, THE THIRTEENTH DAY OF ILLNESS

Although to a less degree, these patients had about the same symptoms as those of the pneumonia group. Severe paroxysms of coughing occurred in several individuals. Nausea, vomiting, dizziness, and epistaxis occurred not infrequently. Moist rales were heard over the lung

fields in 8 cases; sibilant rales were heard in 4 cases. Conjunctivitis and tympanic congestion were present in a few individuals. There was no cyanosis and breathing was easier than in the other group. The temperature usually ascended gradually for two or three days until it reached 100–104 degrees where it remained for a few days before receding. The white blood count followed the course as given in the other group, namely, within or just above normal limits for the first several days with a rise to 12–18 thousand per cmm. as the temperature

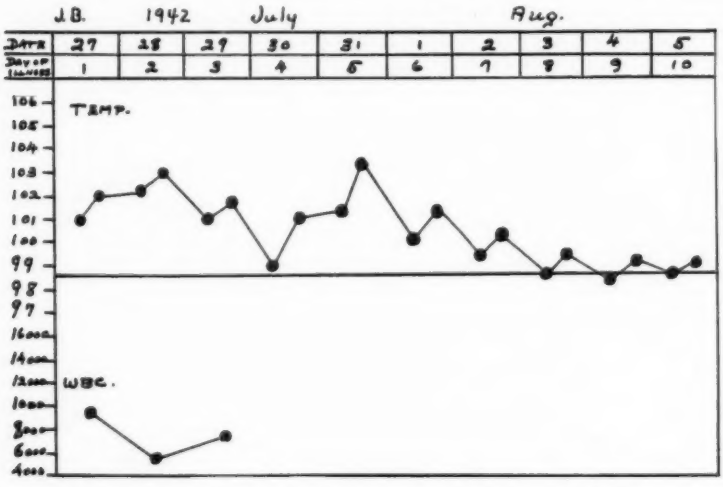

CHART 2. CASE 3. BOY AET. 12. TEMPERATURE CHART AND CURVE OF TOTAL LEUCOCYTES

was returning to normal. As not all of those having rales had roentgenograms taken, it is possible that some members of this group would fit into the pneumonia category.

Case 2. J. F., a ten year old boy, was found to be generally healthy on routine physical examination on July 3rd. On July 27th he was seen because of cough. His throat was not injected and his temperature was 99 degrees. On the evening of July 31st he entered the infirmary because of symptoms of common cold and a temperature of 100.6 degrees. The next day his temperature was normal except for the evening reading which was 100 degrees. He was entirely afebrile the third day and was discharged that evening. He remained entirely well during the remaining four weeks of camp.

Case 3. J. B., a 12 year old boy was admitted to the camp infirmary on July 27th complaining of cough and fever. His July 3rd routine physical examination had been negative. He developed coryza without fever on July 17th. Three days later he started out on a canoe trip with a group from camp and on July 21st he complained of sore throat. According to the councillor in charge, he had a temperature of 99 degrees and did not appear ill. Cough, fever, and general malaise appeared on July 27th and he was returned to the main camp. His temperature of 102 degrees on the first evening reached 103 degrees on the second day and 103.2 degrees on the fifth day of infirmary stay. The temperature curve was swinging in type and gradually returned to normal by the seventh day. Examinations revealed nothing to account for the fever except cough and a slightly injected pharynx. Roentgenograms were not taken.

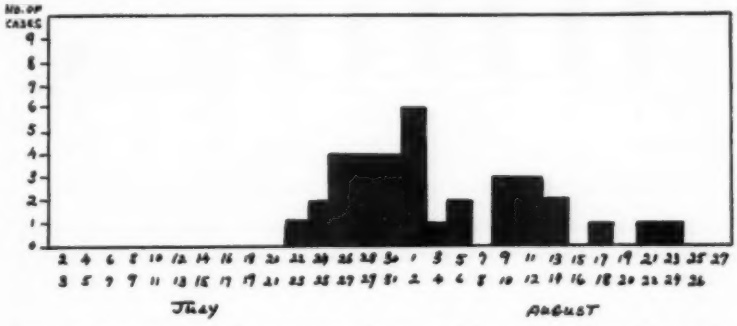

CHART 3. INCIDENCE OF RESPIRATORY DISEASE DURING THE CAMP PERIOD OF EIGHT WEEKS

Each case is charted on the day fever began

EPIDEMIOLOGY

Included in this analysis are those cases of respiratory disease in which at least cough and fever were present. The mildest case had cough with fever lasting two days and the most severe case had roentgenographic evidence of atypical pneumonia. During the eight weeks, eight individuals were taken ill with chicken pox.

Incidence

On chart 3 are placed the 35 cases of respiratory disease according to the day which fever began in relation to the entire camp period. It can be seen that the peak of incidence is one month after the camp opened. The seven cases of atypical pneumonia are scattered irregularly between July 22nd and August 14th.

Age and Housing

Charts 4 and 5 show that the epidemic had its higher rate of incidence among the younger boys and in the dormitory rooms containing the

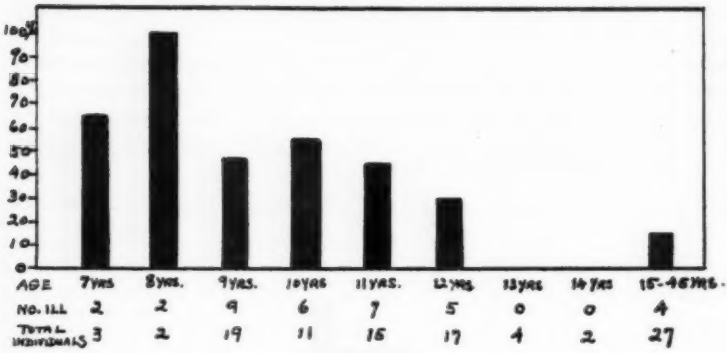

CHART 4. INCIDENCE OF RESPIRATORY DISEASE ACCORDING TO AGE

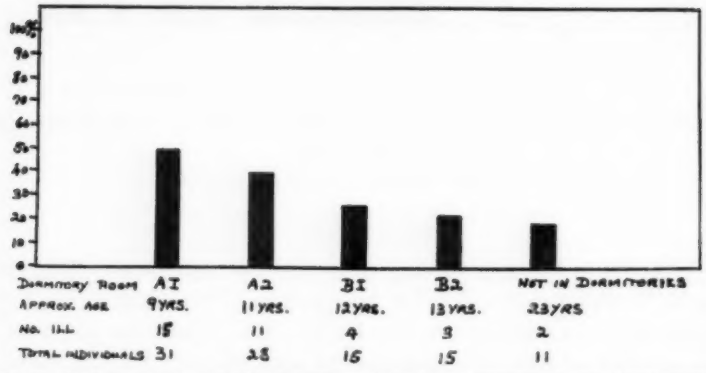
DORMITORY ROOM A1 A2 B1 B2 NOT IN DORMITORIES
APPROX. AGE 9YRS. 11YRS. 12YRS. 13YRS. 23YRS
NO. ILL 15 11 4 3 2
TOTAL INDIVIDUALS 31 23 15 15 11

CHART 5. INCIDENCE OF RESPIRATORY DISEASE ACCORDING TO DORMITORY ROOMS

The incidence was higher in the rooms occupied by the younger boys than in those occupied by the older ones

lower age groups. It cannot be determined whether age or housing played the greater role. There appeared to be greater intermingling among the younger boys. Also, the first cases were found to occur in the

younger group. Although there was a tendency for boys having beds near each other to become ill at about the same time, this did not occur regularly. There was opportunity for general spread of the disease in the common dining hall, game room, and waterfront because of no set arrangement for the boys.

TABLE 1

NAME	DATE OBSERVED	CONDITION
M. T.	July 6	Cough
W. T.	July 10	Cough
E. A.	July 10	Coryza
R. K.	July 19	Cough, fever, and rash
A. F.	July 22	Atypical pneumonia
T. S.	July 25	Cough and fever
G. S.	July 25	Cough, fever, and rales
R. C.	July 25	Convulsion, near drowning, and fever
R. N.	July 26	Atypical pneumonia
J. F.	July 31	Cough and fever
R. A.	August 9	Cough and fever
A. T.		Negative

These twelve individuals left camp on July 7, five days after camp opened. They camped out as a unit for four days and then returned to the main camp. Three individuals had symptoms of common cold during the trip. Six boys were taken ill with the respiratory disease between nine and thirty days after returning to camp. Two of these cases showed roentgenographic evidence of atypical pneumonia; they became ill between the 12th and 16th day after returning to the camp. They were the first cases to develop pneumonia in the entire camp group. R. K. is not included with the cases of respiratory disease in this paper because of rash which was not observed in the others.

Mode of Spread

Presumably the respiratory disease was spread by contact. Deep well water and pasteurized milk were used and there was excellent refrigeration of perishable food. There was no evidence that birds or insects were agents of transmission. There were modern toilet facilities. A high incidence of disease was observed in a group of 12 individuals who were in very close contact for four days early in the camp season. This is shown in Table 1, which also suggests a long incubation period for the disease.

SUMMARY

1. A record is made of an epidemic of atypical pneumonia associated with acute respiratory disease.

2. Clinical and epidemiological material is recorded.

3. Evidence is presented that strongly suggests that primary atypical pneumonia, etiology unknown and acute respiratory infection of mild or severe degree are manifestations of the same disease.

Grateful acknowledgment is made to Dr. Warfield T. Longcope and Dr. Alexander D. Langmuir for their generous help in the preparation of this paper.

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SKIN SENSITIVITY TO MYCOBACTERIAL TUBERCULINS IN SARCOIDOSIS

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The literature on Besnier-Boeck's disease abounds with experiments and discussions designed to propose or to prove the relation of this baffling condition to tuberculosis. However, little work has been done on the possible etiologic role of other mycobacteria. The object of this report is to consider the question: Is sarcoidosis a mycobacterial disease?

The cellular reaction in sarcoidosis suggests some form of mycobacterial disease. It is like that seen when a resistant host is inoculated with an avirulent acid-fast organism (Smithburn, 1937); that is to say, a species of animal which is highly resistant to a strain of acid-fast organism virulent to some other species limits the bacilli to the production of hard tubercles without caseation.

Of similar interest is the statement of Longcope and Pierson (1937): "It is important to recall the work of Sabin, Doan and Forkner on the reactions of the connective tissues of normal animals to the lipoids of the tubercle bacillus. A phosphatide fraction A-3 was particularly potent in calling forth the production of epithelioid cells and giant cells which were frequently arranged in tubercle formation. When the injections of phosphatide were stopped, the tuberculoid tissue underwent resolution and gradually disappeared. Some of the illustrations showing the effects of the phosphatide A-3 are not unlike the lesions encountered in Boeck's sarcoid."

It is, of course, widely believed that in sarcoidosis there is a marked hyposensitivity to Old Tuberculin (i.e. prepared from the fixed mammalian types of tubercle bacilli, human and bovine). Patients having sarcoid may be anergic to large quantities of this tuberculin (i.e. 10–50 mgms.). An interesting report is that of Lemming (1940) who failed to produce skin sensitivity to O.T. by inoculating a patient

who had sarcoidosis with 0.05 mgms. of viable B.C.G.—a procedure which in the normal individual will cause the development of a positive reaction after 2–4 months.

Interestingly enough Kissmeyer (1932) found a positive tuberculin reaction, usually quite weak, in about 40 per cent of reported cases of sarcoidosis tested with O.T. This figure is possibly less than that obtained when any group of patients of a similar age without known tuberculosis is tested. But the difference is not great. Many authors, Longcope (1941) and others, report a lower percentage of positive reactions, and he and Schaumann (1924, 1934) both have shown that patients having sarcoid who react negatively to O.T. may become positive to O.T. later when proven pulmonary tuberculosis occurs.

If sarcoidosis is due to any of the mycobacterial group besides M. tuberculosis, one would perhaps expect some hypersensitive manifestation in the skin of these patients when tested with the growth products of the bacillus concerned, prepared as "Old Tuberculins". One can find, however, few experiments testing the reaction of these patients to protein, fat, or carbohydrate products of other mycobacteria. Herein are reported skin sensitivity tests in seven cases of sarcoidosis. These tests were designed to give evidence for or against specific members of the mycobacterial family as etiological agents. The "tuberculins" used were prepared after the original Koch method for O.T. The organisms used are listed in Table I.

CASE HISTORIES[1]

Seven negro patients having sarcoidosis were tested with mycobacterial tuberculins. In five of the seven, lymph node biopsies made shortly prior to the tests had revealed microscopic evidence of sarcoid. In the other two cases biopsies of lesions had been made many years before; these were at first classified as non-caseating tuberculosis, but the later clinical course and review of the pathological sections led to a revised diagnosis of sarcoidosis. In no patients have tubercle bacilli been found, either by smear, guinea-pig inoculation, or by culture.

[1] Most of these patients have been described by Longcope in his papers on Besnier-Boeck's disease, Longcope (1937, 1941).

TABLE I

Skin Reactions to Various Mycobacterial Tuberculins in Patients Having Sarcoidosis

	PATIENTS													
	T. M.	R. G.			J. C.		D. D.		H. S.		E. M.		W. P.	
	Dilution of tuberculin													
	1/10^{8}	1/10^{8}	1/10^{5}	1/10^{2}	1/10^{2}	1/10^{5}	1/10^{2}	1/10^{5}	1/10^{2}	1/10^{5}	1/10^{3}	1/10^{5}	1/10^{3}	1/10^{5}
1. M. mist....	0	0	0	0	0	0	0	0	0	0			0	0
2. M. berolinensis...	0	0	0	0	0	0	0	0	0	0			0	0
3. M. butter of Rabinowitch...	1+	1+	0	1+	0	0	0	0	0	0			0	0
4. M. butter—Korn.....	0	1+	0	1+	0	0	0	0	0	0			0	0
5. M. stercusis	1+	0	0	1+	0	0	0	0	0	0			0	0
6. M. milk....	0	0	0	0	0	0	0	0	0	0			0	0
7. M. pseudoperlsucht.....	1+	0	0	0	0	0	0	0	0	0			0	0
8. M. marinum #2	1+	1+	0	0	0	0	0	0	0	0			0	0
9. M. chelonei	1+	1+	0	0	0	0	1+	0	0	0	0	0	0	0
10. M. leprae "L_i".....	0	0	0	0	0	0	0	0	0	0			0	0
11. M. smegmatis, H.P.I....	1+	3+	0	2+	0	0	1+	0	0	0	0	0	0	0
12. M. thamnopheus #1.....	0	0	0	0	0	0	0	0	0	0			0	0
13. M. tbc. H-37.....	1+	5+	1+		0	0	0	0	0	0	4+	2+	0	0
14. M. phlei....	—	1+	1+		0	0	0	0	0	0			0	0
15. Control.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16. Chromogenic.....	0	0	0	0	0	0	0	0	0	0			0	0
17. M. Johne...		1+	1+	1+	0	0	0	0	0	0	2+	0	0	0
18. M. of cattle skin lesions....	1+	1+	0	1	0	0	0	0	0	0	0	0	0	0
19. M. tbc. avian....	1+	1+	0	1/10 2+	0	0	0	0	0	0	0	0	0	0

Case I. T. M. (#10436), born 1911, was first seen in 1925, when he complained of slowly progressive, painless swellings behind each ear. A roentgenogram at the time showed a large mediastinal gland with some hilar consolidation. A biopsy of cervical and axillary glands was regarded as representing tuberculosis without caseation. The patient went to the country after an ineffectual course of ultraviolet radiation and was not seen again until 1930 when he appeared with an eruption on the back of his neck. There were also yellow, eroded, palatal lesions present. The Wassermann was 4 plus and the patient was treated until he became sero-negative. A roentgenogram in 1930 revealed consolidation in the hilum of the right lung. Arsenical therapy did not affect the skin lesions.

In January 1936, Dr. Longcope noted phalangeal lesions visible on inspection and by x-ray. The nails of the hands were distorted and thickened, and there were small elastic nodules about the phalangeal joints. On the skin of the face, arms, neck, and back there were pale, flat, rounded lesions. There was inflammation of the left conjunctiva of a year's duration and left ear deafness. The previous diagnosis was reconsidered and the condition was regarded as Boeck's sarcoid. In April 1937 as much as 0.1 mgm. O.T. failed to give a positive reaction.

Case II. R. G., born 1900, Unit #9440, was first seen in 1926 complaining of a nodule below the angle of the left jaw, which for a year had gradually increased in size, and of inguinal swelling. There was general glandular enlargement which subsided early in 1927 after ultraviolet radiation of the enlarged glands. A roentgenogram showed large mediastinal glands with infiltration suggesting Hodgkin's disease. Blood eosinophiles varied between 12 and 15 percent. A biopsy (S.P., #40411) of a cervical node was interpreted as representing tuberculous lymphadenitis without caseation.

In April 1928, she was seen in the department of gynecology because of menorrhagia and there a diagnosis of tuberculous endometritis was made (Gyn. Path. #33267). Roentgenograms now showed diffuse infiltration of both lungs, suggesting miliary tuberculosis. In 1936 roentgenograms revealed the same picture.

In January, 1937, the patient failed to show a response to O.T., 1 mgm., or to avian tuberculin, 1 mgm., after 48 hours, but after five days two raised, pigmented areas appeared, each about 2 cm. in diameter. These were interpreted as delayed reactions. In September 1940 the reaction to 0.1 mgm. of O.T. was 2 plus. In January 1941, the serum globulin was 4.18 gm. The total serum proteins were 7.45 gm.

Case III. J. C., born 1919, #200678, first came to the hospital in May 1940 because of loss of weight since 1936, dyspnoea, oedema and stomach pain. Between March and May 1940, he had lost 20 lbs. He was found to have general glandular enlargement, a palpable spleen and liver, pre-pubertal genitalia, and on roentgenography a contracted right lung and miliary infiltration of the left lung. On June 6, a biopsy of the left inguinal gland was regarded as typical of sarcoid

(S.P., #78053). The patient also gave a history of having had photophobia in 1937, and examination of the eyes revealed evidence of old, broken, posterior synechiae in the right eye with corneal scarring.

In January 1942, he was readmitted in cardiac failure and was discharged in February after treatment. His spleen was not palpable at this time. A roentgenogram in August suggested the possibility of a cavity in the right lung. The reaction to O.T. has been negative since his first admission. He was negative to 10 mgm. in January, 1942. At this time the serum globulin was 4.5 gm., the total protein 8.34 gm.

Case IV. D. D. (#178181), born in 1910, came for the first time to the hospital in August 1939, complaining of abdominal pain after meals and of the presence of a mass in the lower quadrant of the abdomen which had been first noticed the preceding February. Splenomegaly, but no general glandular enlargement was found. A roentgenogram was described as showing tuberculous infiltration of both lungs. Splenectomy was performed and a diagnosis of sarcoid was made by pathological section. Subsequent x-rays continued to give the same picture.

In January, 1941, skin lesions were observed on the patient's upper lip, forehead, and on the back of her neck, although none had been observed on the first visit in August, 1939. These were papular, 8–9 mm. in diameter, with raised annular borders. A skin biopsy, #4251, was interpreted as showing "tuberculosis cutis." In January 1941, the serum globulins were 4.34 gm.; the total serum proteins were 8.22 gm.

Case V. H. S. (#162537), born 1917, was first seen in 1939 because of a low-grade iritis in the left eye. A roentgenogram showed a slight increase in hilar shadows. In January 1942, there were no signs of uveitis, but x-ray revealed infiltration of both lungs, especially of the right middle lobe. Examination of an inguinal lymph node, removed by biopsy, revealed a histological picture of sarcoid. (S.P. #83170).

The patient has two brothers who are known to have sarcoid. The patient was reported as showing a weakly positive reaction to 10 mgm. O.T. in January 1942. At the same time the serum globulins were 3.32 grams, the total serum proteins 7.76 grams.

Case VI. E. M. #191131, born 1902, first came to the Johns Hopkins Hospital in January 1940, because of para-umbilical cramps of a year's duration. Treatment was begun for late latent syphilis and ulcerative colitis. In May 1941, he complained of exertional dyspnoea. In January 1942, he complained of morning cough. In August a roentgenogram revealed diffuse infiltration of both lungs as well as cardiac enlargement. The blood Wassermann was still positive. In September Dr. Longcope saw the patient who then had general glandular enlargement and many small waxy firm nodules which had first appeared in July, 1942, as well as enlargement of the lacrimal glands. A biopsy of the left epitrochlear

lymph gland showed the picture of sarcoid. The serum globulins were 4.05 grams; the total serum proteins were 8.32 grams.

Case VII. W. P. (#252038), born 1917, was admitted to the Medical Service with a definite history of rheumatic fever (St. Vitus Dance and two attacks of migratory polyarthritis). There was a history of 5 years' duration of eye pain, redness and lacrimation with progressive loss of vision. On admission in March 1942, small maculopapular lesions on the thorax and upper abdomen were seen. Cataracts were found in both eyes. There was fibrosis, especially of the right lung, with a pleural effusion on the right side. A diagnosis of rheumatic heart disease with mitral stenosis and insufficiency and auricular fibrillation was made.

TABLE II

Skin Reactions to Various Mycobacterial Tuberculins in Five Normal Subjects

	CONTROL SUBJECT									
	H. S.		R. D.		S. B.		J. B.		R. H.	
	Dilution of tuberculin									
	1/100	1/1000	1/100	1/1000	1/100	1/1000	1/100	1/1000	1/100	1/1000
1. M. chelonei	0	0	0	0	0	0	1+	0	1+	0
2. M. of cattle skin lesions	0	0	0	0	0	0	5+	2+	0	0
3. M. smegmatis	0	0	0	0	1+	0	0	0	3+	1+
4. M. Johne	0	0	0	0	0	0	2+	2+	1+	0
5. M. tbc. H-37	0	0	0	0	0	0	5+	4+	5+	2+
6. Control	0	0	0	0	0	0	0	0	0	0

Total serum proteins averaged 8.38 grams; globulin averaged 4.78 grams. There was 1 plus to 4 plus albumin in the urine. The reaction to as much as 3 mgm. of tuberculin was negative.

A diagnosis of sarcoidosis was made after biopsy and study of an axillary node.

III. EXPERIMENTAL

Eighteen mycobacterial tuberculins and one control preparation were used. These were prepared by the methods used to make Old Tuberculin. Dilutions of 1/1000 and 1/100,000 were used; additional dilutions of 1/100 and 1/10 were used in some instances. The following scale was used to measure the reaction after 48 hours: 0, no reaction, 5 mm. erythema; 1 plus, 5–10 mm. erythema; 2 plus, 1–1.5 cm. erythema; 3 plus, 1.5–2.0 cm. erythema; 4 plus, 2.0–2.5 cm. erythema; 5 plus, 2.5–3.0 cm. erythema.

Five healthy males (white) between 22 and 27 years of age were tested as controls with M. Johne, the mycobacterium of cattle skin lesions (Crawford, 1936), M. chelonei, M. smegmatis (H.P.I.), and with O.T. H–37, i.e. five tuberculins in all; dilutions 1/100 and 1/1000 were used.

DISCUSSION

It will be seen from the foregoing results that these patients with sarcoid did not differ significantly from supposedly normal controls in their response to various mycobacterial tuberculins. Of interest is the fact that 3 out of 7 of our patients (43 per cent) showed positive reactions to O.T. (1:1000). This is higher than was expected, but must be accepted, for it is to be noted that in all of these patients there was positive histological evidence of sarcoidosis and in none of them have true tubercle bacilli been found in smears, by guinea-pig inoculation, or in culture.

In addition to the "Old Tuberculin" prepared from H-37, a well studied strain of M. tuberculosis hominis, other tuberculins of this series which produced positive reactions were the mycobacterium of skin lesion disease in cattle (Crawford, 1936), M. Johne, M. tuberculosis avian, M. smegmatis, and M. chelonei. Admittedly most of these positive reactions were weak.[2] It will be noted that M. leprae, "L_1", produced no positive reactions in this series.

Patients who reacted to human tuberculin, both those having sarcoid and controls, usually showed a slightly positive skin reaction to one or more of the other tuberculins. This is probably a manifestation of the close relationship in the Mycobacterial family, for sensitization to more than one of this group of acid-fast bacilli may occur when an animal is experimentally infected with a specific type.

[2] The vole acid-fast bacillus (Wells, 1937), (Brooke, 1941) and (Wells and Brooke, 1940) was used to prepare a tuberculin for testing these patients. Because of difficulty in growing this bacillus on liquid media, an "Old Tuberculin" preparation could not be made similar to the others used here. Preparation of a tuberculin from the vole bacillus followed the method of Koch's "New Tuberculin." Results with this were unreliable as verified by repetition with two separately prepared tuberculins of this nature, so that no mention of this is included in the experimental section of this paper. In general, however, results with this tuberculin were no different, in the sense of being significantly positive, than in the case of any of the other mycobacterial tuberculins.

In our cases it is likely that these patients acquired their sensitivity to the mycobacterial tuberculins listed above when they became sensitive to tuberculin of the human tubercle bacillus. Most serological work also indicates this chemical-antibody similarity of mycobacteria in vivo (Wilson, 1925), (Furth, 1926). Seibert (1941) has indicated that serological studies show group specificity among acid-fast bacilli to be due to polysaccharides. She believes that type specificity is caused by proteins.

Because most pathogenic members of the mycobacterial family cause hypersensitive skin reactions to the homologous tuberculin, we believe these experiments may be regarded as supporting the view that sarcoidosis is not caused by any mycobacterium in our series.

SUMMARY

1. Eighteen tuberculins prepared from different members of the mycobacterial family were used to test for hypersensitive skin reactions in seven patients with sarcoidosis. Tuberculins giving a positive test were used to study five normal controls. Three of seven patients with sarcoid disease and two of five controls showed positive reactions to "Old Tuberculin", prepared from human tubercle bacilli, and to a few other mycobacterial tuberculins.

2. These experiments offer no evidence for, and in fact represent evidence against, the view that the etiology of Besnier-Boeck's disease is related to any member of the mycobacterial genus used in our series.

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The authors wish to record their indebtedness to Dr. H. J. Henderson of the Henry Phipps Institute in Philadelphia and to Dr. A. B. Crawford of the Bureau of Animal Disease, Beltsville, Md., for the preparations of tuberculins which they so kindly supplied. They would express their gratitude to Dr. Warfield T Longcope and Dr. A. Murray Fisher of the Department of Medicine, Johns Hopkins Hospital for their encouragement and advice.

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MINIMUM MAINTENANCE REQUIREMENT OF AN ENZYMIC CASEIN HYDROLYSATE

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Before protein can be absorbed and utilized, it must be hydrolyzed into its constituent amino acids by gastric and intestinal enzymes. Thus the measurement of the nutritive quality of a protein is basically a study of its hydrolytic products. It follows that the *in vitro* splitting of the protein molecule should not deleteriously affect its nutritive qualities. On the other hand, technical processes for hydrolysis, and for the subsequent handling and processing of hydrolysates will not necessarily duplicate processes within the intestine, so that protein hydrolysates may not always be as nutritive as the original protein (1, 2).

Since hydrolyzed protein products are currently being employed to supply nitrogen nourishment by vein, it is especially important to know that the hydrolysates thus administered have the nutritive qualities desired. Elman (3) employed an enzymic casein hydrolysate (4) for intravenous administration and observed positive nitrogen retention in postoperative cases. A number of similar reports by others have recently appeared (5, 6, 7, 8, 9, 10). Similar positive nitrogen balances in children were observed by Shohl and Blackfan (11) and by Farr, Emerson and Futcher (12). The latter authors reported that intravenously administered casein hydrolysate slightly increased the efficiency of nitrogen utilization in nephrotic patients.

The hydrolysate employed by these investigators was made through the hydrolytic action of the enzymes of pork pancreas (4). During hydrolysis, the protein of the pancreas is also digested, so that approximately 85 per cent of nitrogen in the final product comes from casein and 15 per cent from the pancreas. Comparison of casein and the enzymic casein hydrolysate[1] in rats, and in hypoproteinemic dogs (4)

[1] The preparation employed is known by the trade name of Amigen. It is a dry powder, non-anaphylactogenic, and contains 12 per cent total nitrogen and 7.4 per cent amino nitrogen.

indicated that the hydrolysate was as effective in promoting growth and serum protein regeneration as the original casein. However, it seemed especially desirable to obtain information on the minimum amount of the enzymic hydrolysate required for the maintenance of nitrogen equilibrium in normally active adults. Such information might serve as a guide to the minimum amount necessary for maintenance intravenously.

It is not here necessary to summarize the many reports dealing with the efficiency of protein utilization, but it should be mentioned that Sherman outlined a procedure for determining the maintenance level efficiency of bread protein (13), and Rose and MacLeod (14) compared the minimum maintenance levels of milk, meat, bread and milk, and soybean curd. The latter studied four young women and consistently observed positive balances at an intake level of 0.5 gm. protein per kilo body weight. In general, such studies have indicated that the adult requirement of protein for maintenance was between 0.5 and 0.7 gm. per kilogram.

EXPERIMENTAL

Four normally active and healthy men (aged 28–38) were our experimental subjects. They were observed for two periods of study separated by a one-month interval in which they ate their normal food. Each experimental period consisted of four days' subsistence on a basic diet exceptionally low in protein, followed by four days during which a weighed quantity of the hydrolysate was added to the diet. In the first period the hydrolysate was fed at an intake level of 0.4 gm. per kilo body weight, and during the second period the level of intake was 0.7 gm. per kilo. The hydrolysate was taken at meal time, the calculated amount having previously been dissolved in water or fruit juice.

The diet consisted of foodstuffs low in protein, presented in as palatable a form as possible.[2] "Bread" consisted of starch-butter crackers. Pure sugar candy was the chief energy source; and a vegetable soup

[2] We wish to express our appreciation to Ruby D. Cox for her planning and supervision of the diets; and to J. C. Wallenmeyer for his cooperation in the study.

made from selected vegetables low in protein, the principal source of bulk. A representative menu is as follows:

Breakfast	*Lunch*	*Dinner*
Coca Cola	Vegetable Soup	Vegetable Soup
Starch Crackers	Starch Crackers	Starch Crackers
Apple Butter	Butter Fat	Fried Apple
Butter Fat	Fruit Salad	Apple Cider
	Root Beer	Candy
	Candy	

Representative samples of the food eaten were analyzed for nitrogen and the total daily nitrogen intake calculated, or (during the second period of study) duplicate samples of everything eaten were accumulated, and aliquot portions of the thoroughly mixed sample analyzed. Nitrogen in food, feces and urine was determined daily in duplicate by macro- or micro-Kjeldahl. The distribution in the urine, as ammonia, urea and creatinine nitrogen[3] was determined daily during the second series of observations, but the values are not presented since they were not particularly informative. Fecal collection continued for 24 hours after the end of the experiment. No illness intervened during either period, and the subjects continued their usual tasks. Subject A (age 28, weight 173 lbs.) was most active physically: in addition to all the technical work involved in the experiment, extra hours and week-ends were devoted to construction work. Subject B (age 38, weight 183 lbs.) was engaged in part-time laboratory and desk work; Subject D (age 32, weight 145 lbs.) was a physician in active practice; and Subject C (age 33, weight 253) engaged in laboratory work involving minimum physical activity. In order of decreasing physical activity, we would list Subjects A, D, B and C. During both series of observations, all four subjects complained of lassitude and a feeling of general debility during the third and fourth day of the protein deficient regimen; but reported the prompt disappearance of this feeling after the second day of supplementation with the hydrolysate.

Emphasis was placed on adequate caloric intake and during the two periods of supplementation, these were (in calories per kilo): Subject A, 34.8, 34.8; Subject B, 26.7, 25.7; Subject C, 17.1, 18.1; Subject D, 28.3, 27.4. Only Subject C was markedly deficient in caloric intake.

[3] We wish to express our appreciation to Dr. A. V. Ratcliff, Harold Nenneker, and V. R. Bender for their help in these determinations.

RESULTS

The nitrogen balances for the four subjects are given in the table. In the first experiment the hydrolysate was fed at an intake of 0.4 gm. per kilo per day. During depletion all subjects showed severe nitrogen loss and this was relieved to a very marked extent during supplementation. Subject A, for example, lost 0.1033 gm. N per kg. during the four-day depletion, but this was reduced to almost 1/10, to 0.0118 in

TABLE I

Nitrogen Balances on Four Active Men

During the depletion period of four days, a diet very low in nitrogen was ingested. During the subsequent period of four days, the diet was supplemented with an enzymic digest of casein prepared by action of pork pancreas (4). In Period I, 0.4 gm. (.048 gm. N) of the hydrolysate per kg. body weight was given; and during Period II, 0.7 gm. (.084 gm. N) per kilo. The values are expressed as gm. nitrogen per kg. body weight

SUBJECT		FIRST PERIOD				SECOND PERIOD			
		Intake	Output		Balance	Intake	Output		Balance
			Feces	Urine			Feces	Urine	
A	Depletion	0.0146	0.0186	0.0993	−0.1033	0.0193	0.0190	0.1061	−0.1058
	Supplementation	0.0649	0.0150	0.0617	−0.0118	0.1050	0.0270	0.0825	−0.0044
B	Depletion	0.0118	0.0127	0.0779	−0.0788	0.0157	0.0165	0.0903	−0.0911
	Supplementation	0.0622	0.0107	0.0650	−0.0135	0.0979	0.0151	0.0762	+0.0066
C	Depletion	0.0085	0.0122	0.0681	−0.0718	0.0108	0.0161	0.0885	−0.0938
	Supplementation	0.0568	0.0131	0.0664	−0.0227	0.0943	0.0145	0.0878	−0.0080
D	Depletion	0.0141	0.0166	0.0766	−0.0791	0.0208	0.0211	0.0721	−0.0724
	Supplementation	0.0632	0.0186	0.0626	−0.0181	0.1010	0.0198	0.0783	+0.0029

fact, by the supplement. Similar reductions in the amount of net nitrogen loss was shown by the other three subjects. In total, however, this level of the digest was insufficient for maintenance. In the second experiment, supplementation with 0.7 gm. of the digest per kg. daily was just about adequate for maintenance, since two of the subjects achieved a slight positive balance while two were slightly negative.

A comparison of the fecal excretion during depletion and supplementation is of interest in estimating absorption. Even though the nitrogen intake during the latter period was increased 5 to 9 times, the

amount of nitrogen lost in the feces was not at all increased. It is also pertinent that the urinary nitrogen output during supplementation with the digest was always less than during the preceding period of low dietary intake. This fact, with the fecal data, indicates complete absorption and retention of the hydrolysate.

DISCUSSION

Data on the minimum requirement of protein hydrolysates for maintenance is needed as a basis for their parenteral administration. Especially is this necessary since hydrolytic and technical procedures may deleteriously affect the nutritive quality of hydrolysates as compared with the intact protein. The enzymic casein hydrolysate employed in this study has been previously shown to effect satisfactory growth in rats, serum protein regneration in hypoproteinemic dogs (4) and nitrogen balance in children (11) and adults (5, 6, 7, 8, 9, 10). It might thus be assumed that the minimum for maintenance would approximate that already established for complete proteins, i.e., 0.5 to 0.7 gm. per kilo body weight per day. As shown by the present work, such an assumption would have been correct.

Two levels of the casein hydrolysate 0.4 and 0.7 gm. per kilo were fed to normally active men engaged in their usual laboratory or medical pursuits. The lower intake level was insufficient for complete maintenance, although tissue nitrogen was spared and the magnitude of the negative balance was greatly reduced. In some reports nitrogen losses of such magnitude have been regarded as virtual equilibrium (13). On a nitrogen intake level slightly higher than 0.4 gm. of the hydrolysate, Rose and MacLeod (14) found four young women consistently in positive nitrogen balance. In addition to a higher intake level, the muscular activity of her subjects was probably less than that of ours.

At the level of 0.7 gm. of the hydrolysate, nitrogen equilibrium was achieved. There was no increase in either urinary or fecal nitrogen loss, indicating complete utilization of the hydrolysate.

SUMMARY

Nitrogen balance studies on four healthy men show that the minimum requirement of an enzymic casein hydrolysate for maintenance is between 0.4 and 0.7 gm. per kilo. This indicates its approximate

equivalence with intact protein for maintenance, since maintenance levels of the latter are usually regarded as between 0.5 to 0.7 gm. per kilo.

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PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, January 18, 1943

I. Tuberculosis Among Student Nurses. Dr. Warde B. Allan.

SUMMARY[1]

The speaker reported the results of a survey of the incidence of tuberculosis among the nurses of the Johns Hopkins Hospital Training School for Nurses. The study was started in September, 1934. A total of 293 nurses were observed from that time to February, 1941. In all pupil nurses x-rays of the chest were taken on entry and at the end of each year of their training. Intradermal tuberculin tests were done at the time of entry and again before each pupil nurse graduated. Weight charts were kept. The average age on admission was 22.1 years. The results were as follows: 95 nurses or 32.4 per cent reacted positively to tuberculin on admission and 178 or 60.7 per cent reacted positively at the completion of their training. Eight nurses or 2.7 per cent developed recognizable tuberculosis. Two who reacted negatively at first developed pleurisy with effusion. Four (two who had shown a negative skin test and two who showed positive reactions) developed parenchymal lesions while in training. Two who had shown positive tuberculin reactions developed similar lesions within a year of completing their training.

DISCUSSION

Dr. Victor Cullen: I think the Johns Hopkins Hospital is to be congratulated upon this study. These statistics correspond very closely with what we have found in the State Sanatorium where we have been training nurses. Since 1912, 2.6 per cent of the nurses have developed reinfection type tuberculosis. These developed tuberculosis in the institution. I am not including those who came in with tuberculosis and had relapses. Dr. Todd reported on the nurses in the University of Georgia Hospital who developed reinfection type tuberculosis. In one class of 21 white nurses, 3 developed reinfection type tuberculosis. In a class of 24 colored nurses, 2 developed reinfection type tuberculosis. All of these at first showed negative tuberculin reactions but developed allergy by the end of the first year. Dr. Schwartz of Saranac reported on 270 nurses. His statistics are much higher than the statistics at Hopkins. All of the graduate nurses showed positive tuberculin reactions. 77 per cent of the student nurses showed positive tuberculin reactions. 75 per cent of the negative reactors became positive and 2.3 per cent of the tuberculosis negative reactors developed reinfection type tuberculosis. 2.1 per cent of the positive reactors developed pulmonary tuberculosis. The statistics

[1] The full report will appear in a later issue.

given by Dr. Allan and the statistics we have in the State Sanatorium are not much higher than those for the general population. These figures are important for all hospitals, since compensation laws are going into effect. When people come with negative tuberculin reactions and develop positive tuberculin responses and reinfection type tuberculosis, the institution is responsible for them and has to pay them. If they come with positive tuberculin reactions and develop tuberculosis, the institution does not have to pay them. If they had tuberculosis and then recovered somewhere else, when they break down and develop clinical tuberculosis in the institution they do not receive compensation. We would like to have an x-ray taken of every person who comes in the Hospital. This is being done at the University of Minnesota. Since then there has been a reduction of 20 per cent in nurses developing tuberculosis. In the University of Chicago Lying-In Hospital, roentgenograms were taken of 11,000 women who came for delivery. One in every 100 had active tuberculosis, but not one had any symptoms. This gives us something to think about. The person who comes to work in the Hospital ought to be tested with tuberculin or roentgenograms of the chest ought to be taken.

Dr. Longcope: I wonder whether the incidence among student nurses is very much higher than among factory workers.

Dr. Allan: I have not seen any results among factory workers or the public at large. The data we have are from a select group. The public at large has never been examined. An effort is being made now to carry out tuberculin tests routinely on the people who come into one of the dispensaries of this Hospital.

Dr. Longcope: Can you get a survey, Dr. Cullen?

Dr. Cullen: It is hard to get a survey. Most of the factories are highly unionized and the workers do not want an x-ray taken before they are employed. They say that if a person is found to have tuberculosis, that person is discharged. The incidence among young factory workers should be about the same as at Hopkins.

Dr. Chesney: Have any studies been made of medical students?

Dr. Allan: No good statistical studies of medical students have been published.

Dr. Chesney: Has there been a reduction in the number of cases developing in the past 20 years?

Dr. Cullen: About 20 years ago the Metropolitan Life Insurance Company made a survey. At that time the incidence was about 12 per thousand. In 1941 it was down to 0.7 per thousand. Not a single new case developed last year. One would expect the incidence to diminish because of the marked lowering of the death rate, which dropped 60 per cent. We are advising all colleges to take x-rays of students. Every student at the University of Maryland, Goucher, and Western Maryland will be examined by roentgenography. Studies by the U. S. Army on draftees are being made and in about a year or so, we will have something of value.

Dr. Rich: The most important practical point in a study of this type is the decision whether or not the exposure to patients with tuberculosis constitutes a hazard for the women who engage in nursing. There is little information regarding the incidence of tuberculosis in female factory workers, but it is important that even if it were shown that the incidence in nurses is the same as that in factory workers,

that would really mean that tuberculosis takes too high a toll among nurses; for there is, of course, a close parallelism between economic level, living conditions and the incidence of tuberculosis; and nurses, in this institution at least, represent a higher economic level and have better living conditions than most factory workers. The incidence of tuberculosis among them should, therefore, be lower than among factory workers, if there is no specific tuberculosis hazard in their occupation. What is needed is, rather, a comparison with young women of similar age and economic status who are engaged in pursuits that do not expose them to tuberculosis but which require a similar degree of energy expenditure.

The low incidence of positive reactors to tuberculin, as found in this and other recent surveys, is worth emphasizing. In the early part of the century, because of the results of certain tuberculin surveys and autopsy studies, the dogma became established that all individuals become infected by the time adulthood is reached. There were, however, surveys in Europe showing only about 50 per cent positive reactors, and Dr. Hamman and Dr. Wolman found approximately that percentage of positive reactors among non-tuberculous adults in the dispensary here some thirty years ago. Tuberculin surveys have been carried out on thousands of young adults in recent years, and the average incidence of positive reactors has been around 50 per cent. Nevertheless, the idea that 100 per cent of all adults are tuberculin positive has become so deeply rooted that one still meets competent clinicians who believe that the tuberculin test is of no diagnostic value in adults because "all adults react anyway". It is true that the subjects of practically all of the recent adult surveys have been university students and nurses, a selected group. A survey of dispensary type adults is at present being carried out here.

Finally, surveys of this type are important for the light they may throw upon the pathogenesis of adult tuberculosis. It has long been believed all over the world that the adult type of tuberculosis is due to the action of resistance acquired through a previous childhood infection. A first infection in an animal has the characteristics of tuberculosis in infants or young children, but if the animal has been previously infected or vaccinated, a test infection exhibits the characteristics of the adult type (slower progress, more fibrosis, inhibition of spread to regional nodes, inhibition of generalization). When it was believed that all persons became infected before adulthood it seemed reasonable to attribute the characteristics of the adult disease to the influence of the resistance acquired from the primary infection. We pointed out several years ago that the results of recent tuberculin surveys may require a revision of that seemingly well established explanation. Since the evidence indicates that a large percentage of the young adult population have not been infected, we should now be seeing many cases of childhood type disease in young adults who become infected for the first time in adult life. This, however, is not the case. Childhood type tuberculosis in white adults is just as great a rarity as it always has been. If further autopsy studies support those of Carnes recently carried out here, we shall have to consider seriously the probability that adult type tuberculosis may be due not to the influence of previous infection, but to an age-determined change in bodily reactivity which permits the adult to react

as he does even to a first infection. It is interesting that Dr. Allan has shown us two cases in which the pulmonary lesion developing in previously tuberculin negative nurses was of the typical adult type. Numerous other studies exist in which the same has been observed.

Dr. Cullen: May I ask Dr. Rich a question? Do you see primary tuberculosis in adult colored people?

Dr. Rich: We do indeed, Dr. Cullen. It is common. We encounter typical infantile type tuberculosis even in adult negroes who have previously arrested a primary infection, which remains as a calcified pulmonary and lymph node complex. It seems quite clear that the native resistance of many negroes is lower than that of the white, and that they cannot develop or maintain acquired resistance as well as the white. This, of course, is excessively rare in the white.

Dr. Fisher: I want to ask Dr. Allan how many of the nurses who developed tuberculosis showed positive and how many showed negative tuberculin reactions. Could he tell from his data whether a patient showing a positive tuberculin reaction is more or less apt to develop tuberculosis?

Dr. Allan: Out of four of the eight who showed negative tuberculin reactions, two developed pleurisy and two developed typical tuberculosis lesions. I did not want to mention this in the discussion because I did not think I could draw any conclusions in such a short period.

II. Studies on Motility of Terminal Small Intestine and Some Conditions Causing Delayed Emptying. DR. LAY MARTIN.

SUMMARY

Roentgenographic studies of the small intestine were made on a series of one hundred and fifty patients, one hundred and twenty of whom complained of symptoms which suggested the gastro-intestinal system as their origin. They were divided into three groups:

1: those in whom barium was retained in the terminal ileum at least fourteen hours after ingestion and who had not undergone appendectomy. 65 cases
2: those in whom barium remained in the terminal ileum at least fourteen hours after ingestion and who had previously undergone an appendectomy. 35 cases
3: those patients whose small intestine was free of barium when examined fourteen hours after previous ingestion of the salt. 50 cases, twenty-five of whom had had previous appendectomies.

It was noted that a large number of patients of Group 1 did not give a history or physical findings suggestive of disease of the appendix or the cecum or of inflammation in or adhesions about the terminal ileum. About one half of this number retained barium in the terminal ileum after they had partaken of breakfast. Of the thirty-five who had had previous appendectomies, fifteen presented no clinical evidence of lower right quadrant adhesions nor inflammation of the ileum.

In the third group there were three patients suspected of having appendiceal

disease, two of having lower right adhesions and twenty in whom the basis of the symptoms was considered psychogenic.

No discussion.

III. A Demonstration of Vascular Innervation. DRS. O. R. LANGWORTHY AND LUIS ORTEGA.

SUMMARY

The nerves in the iris of the albino rabbit were stained vitally with methylene blue. The entire membrane was removed, fixed, cleared and mounted upon a slide in one piece. Under these conditions it was possible to observe the course of the nerves to their terminations, and to study them with all powers of the microscope. Nerves in the arterial wall were seen to end in bulb-like terminations close to the nuclei of smooth muscle cells, and also in contact with the cells of the endothelial layer. The capillaries were abundantly supplied by unmyelinated nerves. It was possible to observe these fibers ending in close contact with the nuclei of the endothelial cells in the capillaries. Myelinated nerve fibers ended in complicated terminations upon the walls of groups of capillaries near the pupillary margin. These myelinated fibers are derived from the trigeminal nerve, and are believed to be sensory. Removal of the superior cervical sympathetic ganglion in the neck caused a degeneration of only a small portion of the nerves to the blood vessels of the iris. In order to denervate the iris, it was necessary to section the entire nerve supply to the iris.

No discussion.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Gynecology and Female Endocrinology. By EMIL NOVAK. 605 pp. Illus. $10.00. Little, Brown and Company, Boston, 1941.

This is a truly modern textbook of gynecology. Gynecology is presented from the pathological, clinical and endocrinological standpoint. The technique of surgical operations is entirely omitted. Since the book is intended for medical students and practitioners this omission would seem a wise one. It is the opinion of the reviewer that medical students in the past have wasted much valuable time observing operations and studying technical details which can be of no value to a large majority of them. Pathology receives more attention than in most textbooks, thus greatly simplifying and making intelligent the discussion of clinical symptoms and treatment which follows. Endocrinological conditions as encountered in gynecology are discussed clearly by the author, who possesses an unusual knowledge of the literature in this field. Gynecological organotherapy is considered briefly and the present status of this ever-changing subject is fairly given. In the opinion of the reviewer, the chapters on ovarian tumors are the outstanding portion of the book. The illustrations, many of which are new, are, for the most part, excellent and well reproduced. The colored photographs are particularly vivid and natural appearing. On the whole the student who studies this text will be given the impression that gynecology is a broad science rather than a specialized branch of surgery.

R. W. T.

Ophthalmology and Otolaryngology. Prepared and edited by the Subcommittees on Ophthalmology and Otolaryngology of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 331 pp. Illus. $4.00. W. B. Saunders Company, Philadelphia & London, 1942. (Military Surgical Manuals, Vol. 2.)

Our colleagues in the Army and Navy are frequently confronted with problems in diagnosis and in treatment which in civil practice would be routinely referred to specialists in the various fields. Expert advice often is not available under war conditions. This is true to an even greater extent in the present World War than it was in World War I, because our forces are more widely dispersed. The National Research Council has realized that this problem exists and has arranged for the publication of short manuals covering several specialties. This volume adequately serves its purpose in the special fields of Ophthalmology and Otolaryngology.

In the section on Ophthalmology are eight short chapters which contain essential information regarding routine examinations of the eyes, acute visual disturbances,

acute ocular inflammations, ocular injuries, medical ocular treatment, local anesthesia and surgical procedures. These chapters have been prepared by members of the Subcommittee on Ophthalmology: Harry S. Gradle, William L. Benedict, Sanford R. Gifford, Peter C. Kronfeld and Lawrence T. Post. William F. Hughes and Alan C. Woods have collaborated on the treatment of war gas injuries. The illustrations, of which there are seventy-three, are in black and white. They have been carefully selected and really illustrate. This reviewer is of the opinion that the volume will fully meet the requirements as visualized by the excellent clinicians who prepared it. It contains information of particular importance regarding treatment which is not available elsewhere in a single volume. It should be read with interest by practicing ophthalmologists.

This reviewer is not competent to state an opinion regarding the section on Otolaryngology. Doubtlessly, it has the same standard of excellence as that already passed upon.

F. B. W.

Chemistry and Physiology of the Vitamins. By H. R. ROSENBURG. 674 pp. Diagrams. $12.00. Interscience Publishers, New York, 1942.

Advances in the field of nutrition have been seven-leagued during the past decade, and a comprehensive review of all aspects of the vitamins is a gigantic undertaking. The present volume represents such an achievement.

The author begins the book with his definition of a vitamin. The latter part of the definition of the vitamins reads: "... do not furnish energy and are not utilized as building units for the structure of the organism, but are essential for the transformation of energy and for the regulation of the metabolism of structural units." It is questionable whether this concept will satisfy many biochemists. As components of catalysts, there seems little reason for visualizing them as separate and apart from "structural units." To those substances which do supply energy or act as structural building units, and are essential for the transformation of energy and the regulation of the metabolism of structural units, the author has given the term "vitagens." The essential fatty acids, essential amino acids, certain carbohydrates, choline and certain sulfur-containing groups are included in this category.

The physiological action of the vitamins in man, experimental animals, plants and microorganisms is discussed. This book would be useful to anyone interested in any aspect of vitamin research. It is the most complete single volume devoted to this group of substances. There is an excellent bibliography, especially of references to papers from European sources. The appendix includes a list as well as abstracts of patents pertaining to the vitamins.

A separate chapter is devoted to each vitamin. Following a tabulation of the nomenclature and chronology, the occurrence, isolation, and properties are presented. The chemical nature of each vitamin, where known, synthesis and industrial methods of preparation follow. Attention is given to the biogenesis and specificity of the vitamins. Chemical, physical and biological methods for the determination of the vitamins are given—clinical methods are likewise included.

The symptoms of the avitaminoses, hypovitaminosis and normal requirements of the vitamins are discussed for man and animals.

J. B. M.

Immunization to Typhoid Fever. By J. F. SILER [and others]. American Journal of Hygiene Monographic Series, No. 17, September, 1941. 276 pp. Illus. $2.50. Johns Hopkins Press, Baltimore, 1941.

The contents of this volume are summarized in the following words on the title page: "Results Obtained in the Prevention of Typhoid Fever in the United States Army, United States Navy, and Civilian Conservation Corps, by the Use of Vaccines: Influence of Antigenic Structure and Other Biological Characters of *E. typhosa* on the Production of Protective Antibodies in the Blood of Immunized Individuals: Increases in Protective Antibodies in the Blood Following the Use of a Single Small Dose of Vaccine for Reimmunization Purposes as Compared with the Use of Three Doses." It is a compilation of the results of investigations which have been carried out under the general supervision of Colonel J. F. Siler, Lieut. Colonel George C. Dunham, Major Don Longfellow and Lieutenant G. F. Luippold, in the research laboratories of the Army Medical School. These studies were conducted over a period of 6 years and were carried out with cooperative assistance from members of the staff of the Medical Department Professional Service Schools at the Army Medical Center. No attempt has been made to completely review the medical literature, nor does the volume consider the experience of other countries in regard to typhoid vaccine. This work will stand out as one of the most valuable contributions to medical science by the Army Medical Corps. The studies which are presented are basic in character and upon them depends most of the present day knowledge of the value of this immunization procedure. It is particularly fortunate that these studies were conducted in the years preceding the mobilization for the current conflict. As a direct result, typhoid vaccination of the hugely expanded military forces has presented no problems of importance. This monograph will furnish the point of departure for future researches to improve the methods of artificial immunization against this group of infections.

K. F. M.

A Textbook of Gynecology. By ARTHUR HALE CURTIS. 4th ed. 723 pp. Illus. $8.00. W. B. Saunders Company, Philadelphia and London, 1942.

A number of valuable additions and important revisions have been made in this new edition of Curtis' work. Notably these are to be found in the chapters on pelvic and perineal anatomy, gonorrhea, uterine myomata, carcinoma of the cervix, corrective operations and ovarian tumors.

The author is to be congratulated upon the wealth of sound gynecologic teaching that he has so successfully provided within textbook limitations. The text throughout reflects the discriminating judgment of an experienced teacher, investigator and clinical surgeon. Established facts and widely accepted principles in gynecology are clearly and succinctly presented, while the kaleidoscopic patterns

of endocrinology and organotherapy in this field are properly represented as being in an active stage of evolutionary change. Discussions of controversial subjects leaves one in no doubt as to the author's opinions regarding them, but there is a conspicuous and creditable absence of dogmatism and intolerance of divergent views. Detailed portrayal of regional anatomy and of many operative procedures has been reproduced from excellent artists' drawings in addition to many less ambitious but distinctly helpful illustrations. Appended to the various sections are valuable lists of references to the most important and recent literature upon each particular subject discussed. The index, too, is adequate and well arranged. Indeed, one has no hesitation whatever in recommending this book without reservation to students, practitioners and specialists alike.

E. H. R.

Psychologic Care During Infancy and Childhood. By RUTH MORRIS BAKWIN and HARRY BAKWIN. 317 pp. Illus. $3.50. D. Appleton-Century Company, New York and London, 1942.

This volume written by two pediatricians associated with the New York University College of Medicine emphasizes the need of psychological care in the treatment of children. The book is dedicated to pediatricians and practitioners and covers in broad outlines the different phases of development from infancy through adolescence. It starts with the explanation of the basic terms and concepts and throws light on the mutuality of the child—environment relationship. Though the interest is focused on the reactions and behavior of the child, the role of the parents and their attitude in their relationship to the child are well recognized and stressed as a factor of superior importance.

Two thirds of the book are devoted to the discussion of normal child development and its variations. However, careful thought and consideration are given to a great number of behavior and emotional disturbances in children, which the family doctor encounters so frequently. The authors try to encompass all the multiple ramifications of their subject, ranging from organic brain disease, epilepsy and intellectual retardation to feeding problems and enuresis and from sibling jealousy, rejectedness and insecurity to truancy and stealing.

The comprehensiveness of the material has made it impossible for the authors to dwell with great detail on therapeutic issues. Directions for management and treatments are in conformity with the present schools of thought but tend to be given in general terms. The experienced pediatrician and practitioner may find this book of great help and value but the beginner may have difficulties following the lines of therapy in practice.

T. T.

Surgical Pathology. By WILLIAM BOYD. 5th ed. 843 pp. Illus. $10.00. W. B. Saunders Company, Philadelphia and London, 1942.

In most hospitals the pathological laboratory is situated far from the operating-room. This distance is suggestive of the great gulf that may unfortunately exist

between the surgical and pathological viewpoints. This book bridges that gulf better than any other one-volume treatise on Surgical Pathology. Its popularity is indicated by the fact that it is already in its fifth edition. This edition is extensively revised, is generously illustrated, and retains the strength and clarity of style for which the author is famous. Among the important and timely additions are excellent discussions of burns, head injuries, and wound infections. The rarer conditions have been relegated to small type. The bibliography at the end of each chapter is compact and easily usable, being now arranged according to subjects. This book can be highly recommended to students or any surgeon desiring a clear and easily digestible exposition of the fundamentals of surgical diseases. It should be especially useful to those preparing for the Surgical Board examinations.

G. G. M.

Clinical Anesthesia. By JOHN S. LUNDY. 771 pp. Illus. $9.00. W. B. Saunders Company, Philadelphia and London, 1942.

The author discusses comprehensively all the aspects of modern anesthesiology. The text is well arranged and readable. Outstanding is the unusually complete index which makes for easy reference. Each type of anesthesia is discussed separately. Not only are all the available agents listed, but the details of the administration of each are thoroughly and clearly presented so that they can be followed by even the relatively inexperienced. There are abundant diagrams and photographs illustrating the material of anesthesia and the procedures of administration.

The physiology of anesthesia is presented in such a way that it becomes a part of the description of the various anesthetics, and yet includes all the factors salient for a clinician. The descriptions of local and spinal anesthesia are very complete and there is an exhaustive description of the techniques of administration, reactions by patients, and efficiency of the anesthesia under various circumstances. Intravenous anesthesia is adequately described. The various volatile and gaseous inhalation anesthetics and balanced anesthesia are thoroughly discussed.

The preanesthetic preparation and the postanesthetic care of patients, and the choice of anesthesia have each been arranged graphically to simplify coördination of the various factors of the operation, the drugs used, and the patients' physical status into equations with definite answers. In addition, these same factors are discussed in relation to some of the more outstanding complicating systemic diseases.

The book is complete but not exhaustive. It is at once a text for the student, and an easily used authoritative reference for the specialist in anesthesiology, the surgeon, or the consultant. There are a few statements with which one does not agree, but at the outset the author declares, "The opinions expressed are the result of my experience." This motif is maintained throughout the book, and the bibliography of outside sources is sparse.

J. L. III.